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Volume 43, No. 2/3, 2008

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An Assessment of Environmental Changes in Three Lakes from King’s County (Nova Scotia, Canada) Using Diatom-Based Paleolimnological Techniques

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Paleolimnological approaches using sedimentary diatom assemblages were used to assess water quality changes over the last approximately 200 years in three lakes from King’s County, Nova Scotia. In particular, the role of recent shoreline development in accelerating eutrophication in these systems was assessed. Sediment cores collected from each lake were analyzed for their diatom assemblages at approximately 5-year intervals, as determined by 210Pb dating. Analyses showed that each system has changed, but tracked different ecosystem changes. Tupper and George lakes recorded shifts, which are likely primarily related to climatic warming, with diatom assemblages changing from a preindustrial dominance by Aulacoseira spp. to present-day dominance by Cyclotella stelligera. In addition to the recent climatic-related changes, further diatom changes in the Tupper Lake core between approximately 1820 and 1970 were coincident with watershed disturbances (farming, forestry, and construction of hydroelectric power infrastructure). Black River Lake has recorded an increase in diatom-inferred total phosphorus since about 1950, likely due to impoundment of the Black River system for hydroelectric generation and subsequent changes in land runoff. Before-and-after (i.e., top-bottom) sediment analyses of six other lakes from King’s County provided further evidence that the region is being influenced by climatic change (decreases in Aulacoseira spp., increases in planktonic diatom taxa), as well as showing other environmental stressors (e.g., acidification). However, we recorded no marked increase in diatom-inferred nutrient levels coincident with shoreline cottage development in any of the nine study lakes. Paleolimnological studies such as these allow lake managers to place the current limnological conditions into a long-term context, and thereby provide important background data for effective lake management.

Key words: paleolimnology, eutrophication, Nova Scotia, diatoms, climate change, acidification

Introduction

Many aquatic ecosystems are being threatened by multiple large-scale environmental stressors such as acidification, nutrient inputs, and climatic change. While monitoring changing limnological conditions is essential for proper ecosystem management, most systems lack detailed long-term data which are required to place current limnological conditions into a broader temporal context. Fortunately, these missing instrumental data can often be inferred using paleolimnological techniques (Smol 2008).

Paleolimnology uses the physical, chemical, and biological information preserved in lake sediments to reconstruct past limnological conditions (Smol 2008). By studying changes in biological assemblages and other indicators from sections of dated sediment cores, and comparing these with current limnological conditions, environmental changes can be assessed and put into a long-term perspective. Paleolimnology can be used to track a wide range of environmental conditions including changes in acidic deposition (e.g., Battarbee et al. 1990; Cumming et al. 1994; Ginn et al. 2007a), climate (e.g., Smol and Cumming 2000; Rühland and Smol 2002; Laird et al. 2003; Smol and Douglas 2007), lake depth (e.g., Moos et al. 2005), fish populations (e.g., Sweetman and Finney 2003; Gregory-Eaves et al. 2004), mining effects (e.g., Salonen et al. 2006), urban development (e.g., Meriläinen et al. 2003), nutrient inputs (e.g., Tibby 2004; Reid 2005), as well as contaminant transport (e.g., Donahue et al. 2006). While a wide variety of chemical and biological indicators have been used in paleolimnological studies, diatoms (Bacillariophyceae), a dominant algal group in many freshwater systems, are the most widely used because they are well preserved in lake sediments, are ecologically diverse, and show quantifiable responses to environmental change (Stoermer and Smol 1999).

The majority of studies on eutrophication have focused on lakes with large-scale nutrient inputs (Schindler 1987), however the role of smaller-scale increases in nutrient levels, such as those following moderate shoreline development, are less well studied. Previous paleolimnological investigations of the effect of lakeshore cottage development on trophic status in Canada have focused on lakes in central Ontario, where cottage development has been particularly intense. These studies have addressed concerns of lake managers, such as nutrient inputs from cottages (e.g., Hall and Smol 1996; Wilkinson et al. 1999), taste and odour problems.
These paleolimnological studies have shown a variety of responses by lakes to diffuse inputs of nutrients associated with seasonal cottages. However, to date, no long-term studies have been undertaken on the effect of cottage development in Atlantic Canada.

Nova Scotia, located on the Atlantic coast of Canada (Fig. 1), has approximately 9,400 lakes larger than 1 ha. The province was first settled by Europeans in 1605 and traditional land use has centered on forestry and agriculture, both of which continue today. Due to this relatively long North American settlement history, most lakes have been impacted, to varying degrees, by anthropogenic changes. One region of Nova Scotia that has undergone a recent increase in the amount of shoreline development, and the focus of this investigation, is King’s County (Fig. 1). Located on the Bay of Fundy coast and approximately 100 km from the city of Halifax, King’s County has historically been the primary agricultural region of the province with 526 km² (or 25% of the county area) under cultivation, resulting in an estimated benefit of about $378 million to the provincial economy (Statistics Canada 2001). However, since the mid 1980s, the number of seasonal and permanent homes has increased, especially on lakefront properties, and lake managers have expressed concern that shoreline development has affected water quality and may be responsible for algal blooms, excessive macrophyte growth, problems with taste and odour, and changes in fish stocks. Monitoring efforts, however, have only been in place for the past decade and lack information on predisturbance (preindustrial or presettlement) conditions.

While previous paleolimnological studies in Nova Scotia have primarily focused on tracking the effects of acidic deposition (Ginn et al. 2007a, 2007b), other studies have assessed changes in drinking water quality (Tropea et al. 2007), impacts of road construction (Ginn et al. 2008a), and the overriding influence of climatic change (Ginn et al. 2008b). The objectives of this current study were to track long-term environmental changes in three lakes from King’s County, Nova Scotia, that have undergone varying degrees of shoreline development and other watershed disturbances. Before-and-after (or top-bottom) analyses of sediment cores from six other lakes were added to the three detailed primary study lakes to provide a better overall assessment of possible environmental changes in the greater King’s County region. While the level of cottage development is not as high as in central Ontario, these catchments represent some of the highest levels of cottage development in Nova Scotia. In the three detailed studies, we track which environmental stressors have affected the water quality of these systems.
Lake George. Lake George (44°55.0'N, 64°41.9'W, elevation: 231 m above sea level) has extensive development in its catchment, including seasonal cottages and year-round homes, a public access beach, a vacation trailer park, and the waterfront recreation area for the Lake George Provincial Park. The lake has a maximum depth of 9 metres, a surface area of 153 ha, is circumneutral (pH = 6.3) (Table 1), and drains into the Gaspereau River. Forestry is the main historical use of the land in this area (Davis and Browne 1996). Despite having a higher elevation than other systems used for hydroelectric generation in the Gaspereau watershed, Lake George has no history of impoundment.

Black River Lake. Black River Lake (44°58.3'N, 64°22.7'W, elevation: 170 m above sea level) was dammed in 1930 and again in 1950 by Nova Scotia Power to provide hydroelectric power generation. While some cottage development has occurred along the shoreline, the area was traditionally used for farming or logging since European settlement, and approximately 45% of the catchment is currently cleared (Davis and Browne 1996). The lake is relatively large for this area, with a surface area of 668 ha. The lake has a maximum depth of 19 metres, and thermal stratification occurs in the deepest regions. Due to wetlands and coniferous forests in the catchment, as well as drowned trees (due to dam construction), the lake is coloured by chromophoric dissolved organic carbon (DOC = 8.6 mg/L, colour = 65 relative units, 2005 to 2006 means) but is circumneutral (lake water pH = 6.9) (Table 1).

Tupper Lake. Tupper Lake (45°01'N, 64°35.4'W, elevation: 201 m above sea level) is the smallest of the three main study lakes (surface area = 36 ha, maximum depth = 3 m) and the least cottage-developed with few homes in an otherwise forested catchment. In contrast to George and Black River lakes, Tupper Lake drains into the Cornwallis River. The catchment has been logged several times throughout its history, and, in the 1950s, a sawmill (which operated until the early 1970s) was built on the shoreline. Currently, the lake is circumneutral (pH = 6.3) and oligotrophic (total phosphorus [TP] = 7.2 μg·L⁻¹) (Table 1).

Other King’s County Lakes. The six lakes analyzed using the top-bottom (before-after) approach have a range of limnological conditions (Table 1) as well as a variety of development histories. Many of the forests in the region were cut to support the local shipbuilding industry in the early 1900s, which may have included those around these lake systems. Like Black River Lake mentioned above, several other systems in the Gaspereau watershed have been impacted by impoundment for hydroelectric generation. Water levels in Gaspereau Lake were elevated during the 1930s, and again during the 1950s at the same time as Black River Lake. Lumsden Pond was created in 1941 by the flooding of a river valley, and connects to Black River Lake via an 8.4-km long canal, which is controlled by a hydroelectric generating station. The flooding of this river valley submerged a large area, including forests and a road, the remains of which were still visible when Lumsden Pond was drained temporarily in 1996.

The degree of current shoreline development from cottages and permanent dwellings varies in these six lakes. Aylesford and Murphy lakes have moderate to heavy shoreline development, including public beaches, while Little River and Hardwood lakes have few dwellings, with their catchments being primarily forested. Finally, Gaspereau and Lumsden lakes have limited shoreline development, with these systems being used primarily for hydroelectric means by Nova Scotia Power.

Sediment Core Collection

Sediment cores were collected from the nine study lakes in July 2005 using a Glew gravity corer (internal diameter = 7.6 cm) equipped with a 50-cm long Lexan core tube (Glew 1989; Glew et al. 2001). The cores were
sectioned at 0.5-cm intervals using a Glew (1988) vertical extruder, placed in individual Whirl-Pak sample bags, and stored at approximately 4°C. The sediment cores obtained from the three detailed core lakes (George, Black River, and Tupper) were analyzed every 2.0 cm throughout the length of the core, an integrated sample with a resolution of approximately 5 years of sediment accumulation (as established by 210Pb dating). The detailed core analyses provide a full picture of the history of the lake throughout the time period represented by the gravity sediment core (between approximately 100 to 200 years). The remaining six study lakes were processed for before-and-after (or top-bottom) analyses where surface sediments (representing the past few years) were compared with those from a core depth of below 15.0 to 15.5 cm (which were analyzed to infer precottage development environmental conditions). In the case of Aylesford, Murphy, and Gaspereau lakes, a third sample from 25.0 to 25.5 cm was analyzed, which represented conditions approximately 150 years before the primary predisturbance sample at 15.0 to 15.5 cm. Thus, the two predisturbance samples can be used to assess natural environmental changes and directly compare these to changes since cottage development began. From these sediment intervals a snapshot of the environmental changes that have occurred from predisturbance times to the present can be compared.

Diatom Preparation and Analysis

Diatoms were isolated from the sediment using a 1:1 molar ratio of HNO₃ and H₂SO₄, as outlined in Battarbee et al. (2001), and mounted on slides using Naphrax. A minimum of 300 diatom valves were enumerated per sample using a 100X oil immersion objective (numerical aperture = 1.3) on a Leica DMRB microscope equipped with differential interference contrast (DIC) optics. Diatoms were identified to the lowest taxonomic level possible using Patrick and Reimer (1966, 1975), Krammer and Lange-Bertalot (1991, 1997, 1999, 2000), Round et al. (1990), Camburn and Charles (2000), and other references fully described in Ginn et al. (2007c). In addition, the number of chrysophyte stomatocysts was enumerated since an increase in the ratio of stomatocysts to diatom valves can be a useful index of increasing trophic status (Smol 1985).

Radiometric Dating

Sediment samples from each of the three detailed core lakes were dried using a Virtis freeze dryer, and approximately 0.5 g of sediment from each interval was placed in individual plastic test tubes for 210Pb dating following the procedures of Schelske et al. (1994) and Appleby (2001). Radioactive decay was analyzed using an Ortec germanium (Ge) crystal detector for 80,000 s. 210Pb, 214Bi, and 137Cs activities (Fig. 2) were calculated using procedures outlined in Schelske et al. (1994). Sediment core chronologies were calculated using the constant rate of supply (CRS) model (Appleby and Oldfield 1978) using the computer program developed by M.W. Binford (1990, University of Florida).

Statistical Analysis and Inference of Limnological Variables

Relative abundances of diatom taxa were analyzed by principal components analysis (PCA) using the computer program CANOCO v.4.5 (ter Braak and Šmilauer 2002). PCA axis-1 site scores were used to compare the main direction of variation in the diatom assemblages. The diatom-based transfer function for Northeastern North America (NENA) developed by Ginn et al. (2007c) was used with the maximum likelihood model to infer the TP
Fig. 3. Relative abundance of the dominant diatom taxa (>5%) and diatom-inferred limnological variables from Lake George, King’s County, Nova Scotia. Abbreviations: PCA = principal components analysis sample scores; cyst to diatom ratio = ratio of chrysophyte cysts to diatom frustules.
Fig. 4. Relative abundance of the dominant diatom taxa (>5%) and diatom-inferred limnological variables from Black River Lake, King’s County, Nova Scotia. Abbreviations: PCA = principal components analysis sample scores; cyst to diatom ratio = ratio of chrysophyte cysts to diatom frustules. The timing of hydroelectric dam construction (1950), known from historical records, is indicated by the solid line.
Fig. 5. Relative abundance of the dominant diatom taxa (>5%) and diatom-inferred limnological variables from Tupper Lake, King's County, Nova Scotia. Abbreviations: PCA = principal components analysis sample scores; cyst to diatom ratio = ratio of chrysophyte cysts to diatom frustules. The grey zone corresponds to the approximate timing of the onset of disturbance (between ~1800 and ~1820), likely related to logging and farming in the catchment. The solid line indicates the end of the inferred period of catchment disturbance, based on changes in the diatom assemblage.
and pH. The strength and significance of the pH model was similar to other studies (RMSEP = 0.45, $r_{\text{boot}}^2 = 0.88$), while the TP model had a lower predictive ability (RMSEP = 0.46, $r_{\text{boot}}^2 = 0.24$) (Ginn 2006; Ginn et al. 2007a, 2007c). Correlations of PCA axis-1 scores with inferred limnological variables were determined using the computer program SYSTAT v.11.0. Figures of $^{210}$Pb activity were generated using SigmaPlot v.10.0. Relative frequency diagrams of the dominant diatom taxa (>5% abundance) and inferred limnological variables were generated using the computer programs TGView v.2.0.2 (Grimm 2004) and C2 (v.1.4, S. Juggins, University of Newcastle, U.K.), with diatom taxa arranged by their PCA axis-1 species scores.

**Results**

**Lake George**

Diatom assemblages from Lake George were relatively stable from the 1850s to about 1970, during which time the most abundant diatom taxa were the tychoplanktonic *Aulacoseira distans* (Ehrenberg) Simonsen and the planktonic *Tabellaria flocculosa* var. *linearis* J.D. Koppen (Fig. 3). Since about 1972 (core depth = 4 cm), the diatom assemblage has been dominated by *Cyctolletta stelligera* Cleve et Grunow in Cleve and Asterionella ralfsii var. *americana* (>45 μm) Körner (McIntyre and Duthie 1993) (Fig. 3). This notable change is reflected in the PCA axis-1 site scores. While there were minor fluctuations, overall diatom-inferred pH and diatom-inferred TP were relatively stable throughout the core. The ratio of chrysophyte stomatocysts to diatom frustules did not change throughout the length of the core.

**Black River Lake**

The diatom assemblages in Black River Lake record a marked change in species assemblages in about 1950 (core depth = 8 cm) (Fig. 4); before then, the diatom assemblage was dominated by *Aulacoseira* spp., with benthic diatom taxa making a contribution to the assemblage. Since about 1950, the abundance of *Aulacoseira* spp., with benthic diatom taxa, contributed to the diatom assemblage. Since about 1973, the abundant taxa were replaced in dominance by several benthic species, including *Navicula leptostriata* Jorgensen, *Nitzschia* spp., and notably *Achnanthidium minutissimum* (Kützig) Czarnecki. During this time, there was also an increase in the diatom-inferred TP from 4.0 to 5.0 μg·L$^{-1}$ to approximately 8.0 μg·L$^{-1}$. This increase in diatom-inferred TP is strongly and negatively correlated to the PCA axis-1 scores ($r = -0.81$, $p < 0.01$). Since about 1973, these taxa (*N. leptostriata*, *Nitzschia* spp., and *A. minutissimum*) have declined in relative abundance, with the current diatom assemblage being dominated by *C. stelligera* and *F. exigua*, while the diatom-inferred TP has remained at close to 6.0 μg·L$^{-1}$ (measured midsummer TP is currently 7.2 μg·L$^{-1}$). It is interesting to note that, unlike *C. stelligera* and *F. exigua*, *A. irata* was not observed to return to the same dominance in the core after about 1973 as was observed prior to about 1828. Despite the importance of planktonic species, such as *C. stelligera*, the majority of the diatoms recorded in the sedimentary record of Tupper Lake, for about the last 200 years, were benthic, with between 60 and 90% of the assemblage being made up of benthic, pennate taxa (Fig. 5). When comparing the ratio of chrysophyte stomatocysts with diatom frustules, the ratio was found to decrease since preindustrial times, with the decrease corresponding to the observed increase in the ratio of benthic to pelagic taxa in about 1800 (core depth 30.0 cm). This decrease, as in Black River Lake, correlated negatively to the inferred TP level ($r = -0.79$, $p < 0.01$). The diatom-inferred pH increased slightly from a predisturbance value of 6.0 to 6.3 in about 1828.

**Tupper Lake**

The diatom assemblages in the Tupper Lake sediment core have undergone a number of changes during the last approximately 200 years (Fig. 5). Prior to about 1828 (core depth = 22.0 cm), the assemblages were dominated by *Aulacoseira lirata* (Ehrenberg) R. Ross, *C. stelligera*, and *Fragilariforma exigua* (Grunow) Krammer and Lange-Bertalot. In around 1800, the relative percentage of the assemblage made up of benthic taxa increased, however the relative abundances of these dominant taxa did not change at that point. Between about 1828 and 1973, these abundant taxa were replaced in dominance by several benthic species, including *Navicula leptostriata* Jorgensen, *Nitzschia* spp., and notably *Achnanthidium minutissimum* (Kützig) Czarnecki. During this time, there was also an increase in the diatom-inferred TP from 4.0 to 5.0 μg·L$^{-1}$ to approximately 8.0 μg·L$^{-1}$. This increase in diatom-inferred TP is strongly and negatively correlated to the PCA axis-1 scores ($r = -0.81$, $p < 0.01$). Since about 1973, these taxa (*N. leptostriata*, *Nitzschia* spp., and *A. minutissimum*) have declined in relative abundance, with the current diatom assemblage being dominated by *C. stelligera* and *F. exigua*, while the diatom-inferred TP has remained at close to 6.0 μg·L$^{-1}$ (measured midsummer TP is currently 7.2 μg·L$^{-1}$). It is interesting to note that, unlike *C. stelligera* and *F. exigua*, *A. irata* was not observed to return to the same dominance in the core after about 1973 as was observed prior to about 1828. Despite the importance of planktonic species, such as *C. stelligera*, the majority of the diatoms recorded in the sedimentary record of Tupper Lake, for about the last 200 years, were benthic, with between 60 and 90% of the assemblage being made up of benthic, pennate taxa (Fig. 5). When comparing the ratio of chrysophyte stomatocysts with diatom frustules, the ratio was found to decrease since preindustrial times, with the decrease corresponding to the observed increase in the ratio of benthic to pelagic taxa in about 1800 (core depth 30.0 cm). This decrease, as in Black River Lake, correlated negatively to the inferred TP level ($r = -0.79$, $p < 0.01$). The diatom-inferred pH increased slightly from a predisturbance value of 6.0 to 6.3 in about 1828.

**Present-Day versus Predisturbance Assemblages**

Before and after (i.e., top-bottom) sediment core analyses of the remaining six study lakes also showed a number of changes between present-day and precottage development sedimentary diatom assemblages (Fig. 6). Two lakes (Aylesford and Hardwood) have recorded a change in diatom assemblage dominance by *Aulacoseira* spp. to present-day dominance by *Asterionella ralfsii* var. *americana* (>45 μm), although only Hardwood Lake showed a significant decrease in diatom-inferred pH (0.3 units). Murphy Lake showed a decrease in *Aulacoseira* spp. in surface sediments relative to bottom sediments, as well as a concurrent increase in the abundance of *C. stelligera* and *A. ralfsii* var. *americana* (>45 μm), similar
to the assemblage shift observed in Lake George. Diatom assemblages in Lumsden Pond and Gaspereau Lake show a similar trend to those in Black River Lake, with an increase in the relative abundance of *T. fl occulosa* strain III in the upper portion of the sediment archive. Little River Lake recorded no major changes in sedimentary diatom species assemblages as determined by before and after analyses.

**Discussion**

**Lake George**

The changes in diatom assemblages in Lake George (Fig. 3) are consistent with primarily climate-related changes over about the past 110 years. Heavily silicified, tychoplanktonic *Aulacoseira* spp., especially *Aulacoseira distans*, were the dominant taxa and represented as much as 40% of the relative diatom abundance below 4.0 cm (before ~1970). After about 1970 (core depth = 4.0 cm), the relative abundance of *A. distans* decreased markedly, and *A. distans* was replaced by *Cyclotella stelligera* and *Asterionella ralfsii var. americana* (>45 μm) as codominant taxa. While the timing of this *C. stelligera* increase began in about the 1940s, *C. stelligera* became the dominant taxon since the 1980s, and *A. distans* decreased in relative abundance to below 5%. The likely cause of this change is an increasing temperature trend, which results in reduced ice cover and a longer period of lake thermal stratification (e.g., Harris et al. 2006; Ginn et al. 2008b; Rühland et al. 2008). Based on instrumental temperature records from nearby Halifax, mean annual temperatures have increased by 1.5°C since 1870, with a 0.8°C increase in mean summer temperature since 1948 (see Ginn et al. 2008b). Heavily-silicified *Aulacoseira* spp. require more frequent mixed water in order to survive in the photic zone, whereas the planktonic and more lightly-silicified *Cyclotella* spp. can survive in stratified waters and out-compete *Aulacoseira* spp. (Rühland et al. 2008). This 20th century warming trend has been observed in other lakes in Nova Scotia (Ginn et al. 2008a, 2008b), nearby New Brunswick (Harris et al. 2006), and numerous other lakes around the world (Smol et al. 2005; Rühland et al. 2008).

Observations by local residents of algal blooms in Lake George during summers since the mid 1970s do not appear to be due to increased nutrient inputs, based on paleolimnological data, as the diatom-inferred TP has remained relatively stable over about the past 110 years. However, the inferred increased thermal stratification caused by warmer waters may have enhanced the competitive abilities of blue-green algae (Cyanobacteria), which also thrive in warmer, stratified waters (Paerl and Huisman 2008). Modelling studies have also concluded that cyanobacteria have the potential to dominate phytoplankton communities under increased temperature regimes (Elliott et al. 2006). Algal blooms have been reported primarily during the late summer, when the lake is more strongly stratified and warmer.

**Black River Lake**

Sedimentary diatom assemblages from Black River Lake (Fig. 4) show that the system has also been
affected by environmental changes that are most likely a result of changes in the watershed (e.g., forestry and dam construction), which would mask other changes (e.g., climate). The change in approximately 1950 in diatom assemblages and the concomitant increase in diatom-inferred TP coincide with the construction of a hydroelectric dam and major changes in the watershed (Mackay P., personal communication [Nova Scotia Power]). Black River Lake has a history of hydroelectric generation that dates back to the early 1930s when the first dam was constructed (Fig. 4); however, major environmental changes likely began in the late 1940s when drainage from Gaspereau Lake was routed into the Black River system. This change in drainage pattern involved flooding an area that was previously forested (the remains of submerged trees still pose a threat to local boat traffic) and impoundment of the river, and resulted in an increase in both the lake’s surface area and water residence time. The flooding of and changes in land runoff in an agricultural catchment led to increases in the nutrient input. In addition, the decomposition of inundated vegetation, as well as leaching of nutrients from flooded soils, has been shown to cause increased nutrient flux to reservoir systems (Kennedy and Walker 1990). The changed hydrology of the system following its impoundment (e.g., longer residence time) would also have likely changed the nutrient fluxes, enhancing the probability of eutrophication. The shift to the strains of *T. flocculosa* in Black River Lake likely also reflects hydrological changes, since the depths of the water was increased following dam construction (maximum depth is currently 19 m). The observed decrease in the relative abundance of *C. stelligera* likely also reflects this eutrophication as a result of hydroelectric impoundment, as *C. stelligera* is recognized as an oligotrophic taxon (Wunsam et al. 1995; Ginn et al. 2007c), which would be less able to compete under higher nutrient conditions.

When examining the ratio of chrysophyte stomatocysts to diatom frustules, the ratio of cysts to diatoms decreased as diatom-inferred TP increased (Fig. 4). This relationship was strongly and negatively correlated ($r = -0.82, p < 0.01$). This follows the conclusions made by Smol (1985) who suggested that a greater ratio of cysts to diatoms indicates more oligotrophic conditions in Ontario lakes. Our data indicate that the timing of the construction of hydroelectric generating stations, and associated changes in Black River Lake, were linked to this change in the cyst to diatom ratio, and this provides further evidence that the system eutrophied at this time.

**Tupper Lake**

The sediment core from Tupper Lake (Fig. 5) represents the longest history of the three lakes, with the deepest sediments in the core estimated to have been deposited in about 1790. Over this time period, the diatom assemblages have undergone several changes. Around 1820, *Achnanthidium minutissimum* increased to about 8 to 9% relative abundance, which we infer to represent the onset of watershed development, as *A. minutissimum* has been shown to occur following catchment development (e.g., Garrison and Wakeman 2000). The timing of this disturbance may have begun as early as 1800 based on the observed increase in the percentage of benthic taxa and the decrease in the ratio of chrysophyte cysts to diatom frustules (Fig. 5). This approximately 150- to 170-year long watershed disturbance is likely related to several historically known periods of logging followed by the cleared area being used for a series of small farms (Griffiths J. personal communication [Municipality of King’s County]), which likely added nutrients to the lake as shown by a slight (4.0 μg·L$^{-1}$) increase in diatom-inferred TP. In 1922, a small hydroelectric generating station was constructed on the lake (Mackay P., personal communication [Nova Scotia Power]), which likely caused additional disturbances, as well as the approximately 1950 construction of a sawmill to process trees harvested in the catchment. The close to 1970 decrease in *A. minutissimum*, along with the decrease in inferred TP, indicate the system had likely recovered from these disturbances. It is known that the local sawmill closed at approximately this time. Currently the watershed is forested and has very few (-10) seasonal cottages. In addition, most of the diatom taxa have returned to predisturbance abundances with the exception of the *Aulacoseira* taxa.

The diatom assemblages from the Tupper Lake core may also track a warming trend, similar to that described for Lake George. The predisturbance diatom assemblage was dominated by *Aulacoseira* spp. and *C. stelligera*, both of which decreased in abundance during the disturbance between about 1822 and 1970. However, following recovery from the disturbance, the assemblage was again dominated by *C. stelligera*. The lack of a return of the *Aulacoseira* spp. complex (particularly *A. lirata*) can also likely be attributed to increased temperatures in the water body as a result of 20th century warming trends (similar to our discussion of Lake George). However, due to the shallow nature of the system, which undergoes continual mixing throughout the ice-free season, better adaptation to thermally stratified waters cannot explain the dominance of *C. stelligera*, as the lake does not undergo thermal stratification. It is likely that *C. stelligera* dominance is due to less ice cover, which allows *C. stelligera* to persist later into the fall/winter (Smol 1988; Rühland et al. 2008). However, the timing of the onset of climate influence in this system is difficult to assess due to the watershed disturbances described above, which resulted in a decreased abundance of *C. stelligera*, and therefore blurred the timing of the onset of climate warming.

Therefore, based on sedimentary diatom assemblages, we conclude that Tupper Lake has been influenced by both disturbances in its catchment, as well the recent (post-1950) climate warming that has affected the region.
Present-Day versus Predisturbance Diatom Assemblages

In addition to the detailed paleolimnological analyses described above, other environmental changes were inferred using the before-and-after (i.e., top-bottom) approach in six King’s County lakes (Fig. 6). Murphy Lake showed a shift in dominance from a preimpact assemblage of *Aulacoseira* spp. to an assemblage dominated by *C. stelligera*, similar to the shifts described for lakes George and Tupper. This is also further evidence that the lakes of this region have been affected by 20th century climate warming. The increase in *Cyclotella* is not observed in all lakes due to the masking effect by other, more pronounced environmental stressors (e.g., acidification in Hardwood Lake, watershed disturbance in Gaspereau, Lumsden, Aylesford, and Black River lakes). Two lakes (Aylesford and Hardwood) have undergone changes in diatom assemblages that may also reflect the impact of a decrease in lake water pH (Fig. 6), although only Hardwood Lake shows a significant acidification signal. In these lakes, the diatom assemblage changed from dominance by *A. distans* and *A. lirata* to a present-day dominance by *A. ralfsii* var. *americana* (>45 μm). This change has been recorded in other Nova Scotia lakes that contain high (>5 mg/L) amounts of chromophoric dissolved organic carbon and have been affected by acidic deposition (Ginn et al. 2007a, 2007b). These lakes have an acidic preindustrial pH due to the presence of organic acids from dissolved organic carbon with diatom-inferred lake water pH values of 5.7 to 6.0 units. These lakes have become more acidic, likely due to deposition of sulphate from long-range transport (Clair et al. 2001).

Diatom assemblage changes in Lumsden Pond and Gaspereau Lake show similar trends to those observed in Black River Lake, and also likely reflect their history of use for hydroelectric generation. The flooding of a river valley in the case of Lumsden Pond, and the increase in the surface area of Gaspereau Lake following dam construction led to changes in the hydrology of these systems, which seems to have favoured the planktonic diatom *T. flocculosa* strain III, as was also observed in the detailed core analysis from Black River Lake. Based on the diatom assemblages determined in the top-bottom portion of this investigation, it appears that only Little River Lake has not been affected by some form of environmental change in about the last 100 years. However, detailed core analyses might provide further evidence for environmental changes in all of these six systems, as the top-bottom approach provides only a snapshot of conditions from which to interpret diatom-inferred changes.

Recent Shoreline Development

The original goal of this investigation was to determine what (if any) impact recent (post-1970) shoreline development, in the form of seasonal cottages, has had on the water quality of lakes in the King’s County region, several of which are heavily used for these recreational purposes. While diatom assemblages have inferred environmental changes in all but one system in the region over about the last 150 years, the timing and type of assemblage shifts we observed are not consistent with any marked eutrophication trends that could be related to increased shoreline development. Eutrophication, which was inferred to have occurred only in Black River Lake, predates the establishment of cottages. On the contrary, detailed core studies of three of the nine lakes in the region show a trend towards slightly decreased TP in about the last 10 years, a trend that has been described previously in Ontario lakes (Hall and Smol 1996). This trend towards recent decreases in TP can likely be attributed to several factors, including decrease in the runoff of nutrient-rich materials from farms, better lake management by local residents, as well as decreased nutrient availability as a result of recent climate warming (e.g., Hall and Smol 1996).

Conclusions

Despite the fact that this investigation found no evidence of marked recent eutrophication as a result of shoreline development, diatom assemblages have shown that lakes in King’s County have been affected by a variety of environmental stressors over the last one to two centuries. While the sedimentary diatom assemblages recorded changes consistent with climate warming in three lake systems, and no significant environmental changes in one King’s County lake (Little River), based on these findings it appears clear that climate warming has affected lakes in King’s County, as has been documented through other paleolimnological investigations in the Maritimes (e.g., Harris et al. 2006; Ginn et al. 2008a, 2008b). Lack of similar changes in diatom species assemblages in all of the study sites may be due to differences in lake depth (Table 1), or masking by other environmental changes (e.g., landscape disturbance, acidification). In addition to climate as an environmental stressor, hydrological alterations to facilitate hydroelectric generation have resulted in changes in water quality in three lake systems in this region.

Using paleolimnological techniques, we were able to establish precottage development reference conditions for these lakes, which were lacking predisturbance instrumental data. From our results, lake managers will be able to put current conditions into better perspective. These data will facilitate environmental planning in response to future changes in these systems.

Acknowledgments

This study would not have been possible without the assistance of: D. Taylor, Nova Scotia Environment; J. LeBlanc and J. MacMillan, NS Fisheries; D. Poole, Municipality of King’s County; and the field assistance of A. Paul, A. Coombs, and K. Lauersen. We thank two
anonymous reviewers whose helpful comments improved the quality of this manuscript. This study was funded by an NSERC Strategic Grant to JPS, BFC, and Peter Dillon.

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Received: 13 November 2007; accepted: 19 June 2008.
Spatial Distribution and Characterization of Contaminated Soils in Riverbanks of Saint-François and Massawippi Rivers (Southern Québec)

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Soils contaminated with hydrocarbons (C10 to C50) and other contaminants were recently discovered in the banks of the Saint-François and Massawippi Rivers, which are between the municipalities of Eustis and Drummondville (southern Québec). It is probable that this contamination originated from accidental or illegal discharges at the site of the old Eustis mine located near the Massawippi River. The contaminated layers sometimes extend more than one metre in the banks. Unlike water quality monitoring, which resulted in numerous government reports, no studies have been done on the contamination of Saint-François and Massawippi riverbanks or river bed sediments, even though these rivers pass through former industrial and mining areas. This study provides an evaluation of the spatial distribution of the contamination along the riverbank and characterizes the contaminated soils in order to evaluate the concentration levels of hydrocarbons and other pollutants (e.g., heavy metals). The results obtained show that the banks of the Windsor area are more contaminated by hydrocarbons (C10 to C50) than the other sites. The other pollutants (heavy metals, PCBs [polychlorinated biphenyl], and PAHs [polycyclic aromatic hydrocarbons]) indicated low levels of contamination, except for some metals (Cu and Zn). The Ministère du Développement Durable, de l’Environnement et des Parcs uses three generic criteria or levels (A, B, and C) to determine the degree of soil contamination. The levels B and C indicate the presence of contaminants in soil, and these levels have certain usage constraints.

Key words: contaminated soils, hydrocarbons, riverbank, industrial and mining pollution

Introduction

The industrial era was marked by extensive industrial use of lakes, rivers, and streambanks. These environments facilitated the use of water resources, which were virtually inexhaustible for manufacturers and industries (e.g., use of large amounts of water by pulp and paper and chemical industries). In many cases, these activities led to the major pollution and contamination problems found today (Deniseger and Kwong 1996; Marsalek et al. 1997; Walker et al. 2002; Forsythe et al. 2004; Kovacs et al. 2004; Martin 2004; Carter et al. 2006). The growing use of petroleum products in various forms was added to other pollution sources, which can be easily traced (Van Vleet and Quinn 1978; Hoffman et al. 1983; Saint-
Laurent et al. 1995; Larkin and Hall 1998; Cozzarelli et al.
2001; Dror et al. 2001; Nikanorov and Stradomskaia
2003; Armstrong et al. 2005). For instance, anthropic
contamination by polycyclic aromatic hydrocarbons
(PAHs) or polychlorinated biphenyls (PCBs) is easily
recognizable by its high sediment concentration, which
exceeds by far the trace quantities found in nature
(Morton 1983; Christensen et al. 1996; Malawska and
Willkomirski 2000). Also, petroleum products (especially
heavy oils) can be present for extended periods in lake
and streambed sediments (Hoffman et al. 1983; Larkin
and Hall 1998). The presence of hydrocarbons, heavy
metals, and other pollutants (PAHs, PCBs, etc.) in soils
and sediments (riverbanks, lakes, etc.) has a direct impact
on aquatic systems and water quality, particularly in areas
subject to severe contamination. These contaminants
in high concentrations are considered to be substances
that affect wildlife, causing eggshell thinning, tumours,
and other deformities (St-Onge and Richard 1996;
Dumaresq and Parker 2002; Dupuis et al. 2002). Elevated
concentrations of pollutants in the river or lacustrine
environment are known to endanger ecosystems and
introduce a potential risk for human health (CCREM
1986; CCME 1994). For example, heavy metals (lead in
particular) in high concentrations have adverse effects on
human health, through heavy metal poisoning, affecting
the central nervous system and/or acting as cofactors
in many other diseases (Bellinger 1995; Tripathi et al.
2001).

This study focuses on a case of contamination, along
the riverbanks of the Saint-François and Massawippi
Rivers, that was never recorded in government reports
or in scientific literature. This recent discovery led to
the identification of soils contaminated mainly by C10
to C50 hydrocarbons and other pollutants, with the
contamination extending over more than 100 kilometres
of riverbank between the municipalities of Eustis and
Drummondville (southern Québec). The main source of
contamination appeared to be a former mine site (Eustis
mine) that has been closed since 1939 (Ross 1975), but
which now serves as a pulp and paper waste disposal
and storage site. The objectives of this study were to (1)
determine the spatial extent of the contamination and
the main types of contaminants found in the riverbank soils,
and (2) evaluate the concentration levels of the contaminants
along the riverbank at different sites. The analysis was focused on determining the concentrations of hydrocarbons (C10 to C50), heavy metals, PAHs, and PCBs in the contaminated soil, and some physical and chemical properties of the soil profiles (pH, total organic carbon, grain size). The procedures for sampling and laboratory analysis followed the criteria and guidelines decreed in the Québec’s Soil Protection and Rehabilitation of Contaminated Sites Policy from the Ministère du Développement Durable, de l’Environnement et des Parcs
(MDDEP 2007). It is important to evaluate the spatial
distribution and concentration levels of hydrocarbon
contamination and other pollutants in the riverbanks,
which can affect the quality of the surface water and
groundwater. It is known, however, that hydrocarbons
are poorly soluble substances in general, and that these
contaminants accumulate preferably on the surface or at
the bottom of groundwater, according to the density of
the pollutant (BRGM 2001).

Study Area and Water Quality

The study area is located in the Saint-François River
basin, which covers a surface area of 10,230 km2 (MEF
1996), 14% of which is found in the United States. The
Saint-François River is the main body of water in this
vast catchment, and it originates in the Saint-François
reservoir lake, which is located in the Appalachians, and
flows northward to feed into the St. Lawrence River at
Lake Saint-Pierre. The magnitude of industrial, urban,
and agricultural activities in the Saint-François River
basin led the Environment Ministry to monitor water
quality, which resulted in numerous government reports
(Primeau 1992; Laliberté and Leclerc 2000; Leclerc and
Mueldermans 2002; Berryman et al. 2003; Painchaud
2007). The initial water quality control measures
undertaken by the Ministère de l’Environnement, in
particular in the industrial sector, date back to the 1970s;
further government measures were added in 1988 and
aimed at reducing industrial waste emissions into the
air, water, and soil (MEQ 1999). In 1991, there were
30 manufacturing companies likely to release polluting
emissions into the basin’s various rivers (MEF 1996),
including pulp and paper mills, which are considered
highly polluting (Berryman 1996). The government
reports mentioned that the waters of the Saint-François
and Massawippi Rivers were severely contaminated by
industrial and agricultural wastes, though the situation has
improved since the 1990s. The installation of wastewater
treatment plants from 1990 to 2000 (Berryman and
Pelletier 2001) in most of the municipalities along the
Saint-François River and its main tributaries seems to
have helped decrease the concentration levels of several
contaminants detected downstream of Drummondville
(e.g., PAHs, PCBs, fatty acids, resin acids). Government
data on the quality of the water in the Saint-François
river catchment reveal that the areas most affected by
industrial, urban, and agricultural waste are mainly
found downstream of Lennoxville and Sherbrooke.
The decrease in water quality is mainly caused by high levels
of fecal coliform bacteria, total phosphorus, nitrates/nitrites,
total suspended solids, and turbidity. Severe metal
contamination and heavy acidification of the effluents
alongside the mine sites, including the former Capelton
and Eustis sites, were also found (Berryman 1996;
Laliberté and Leclerc 2000; Berryman and Pelletier 2001;
Berryman et al. 2003). Unlike water quality monitoring,
no studies have been done on the contamination of Saint-
François and Massawippi River sediment or soil, even
though these rivers pass through former industrial and
mining areas. Note that the Massawippi River, a major
A tributary of the Saint-François River, flows near a former mining site (Eustis mine) that was in operation from 1865 to 1939 (Ross 1975). During this period, the ore extracted from the site consisted of copper and pyrite (chalcopyrite mineral), and from 1889 to 1927, about 34,000 tons of ore were extracted per year (Ross 1975). At the site, open heap ore roasting was done, and later, large furnaces were used to eliminate a large portion of the sulphur in the ore through combustion followed by melting to increase the copper content (between 40 and 70%). The author (Ross 1975) mentions that the streams received a variety of suspended and dissolved pollutants from discharged mine waters. One can assume that a portion of the mining waste is now part of the Massawippi riverbed. No studies were done to assess the proportion of mining waste in the riverbed, but layers of ore are found buried all over the riverbanks; these layers can easily attain 3 to 5 cm in thickness, and are easily identifiable through a reddish colour or by rust. Like several other former mine sites, the Eustis mine is considered an “orphaned” mine site, which are usually under the responsibility of the Ministère de l’Environnement du Québec. This site currently serves as a pulp and paper waste disposal and storage site, and in 2006, the Québec government announced investments of more than 1.5 million dollars for its restoration.

**Materials and Methods**

**Spatial Distribution of Contaminants**

To identify the probable source of the contamination and assess the spatial distribution of the contaminants along the riverbank, field work was conducted between 2003 and 2006. The study area was divided into five-kilometre-long sections using topographic maps (MRNFP 2000)
and maps of high-risk flood zones (Environment Canada and MEF 1981) over a distance of 103.5 km. The five-kilometre sections were subdivided into one-kilometre sections in order to obtain the best spatial contamination representation. Sampling sites were selected randomly within each kilometre. In the section upstream of Windsor and downstream of Sherbrooke, only two sites were retained due to the predominance of rocky substrate along the riverbank (approximately 50 km of rocky riverbanks between these two municipalities). Site selection criteria also took into account soil materials that showed visual and olfactory indicators of contamination (e.g., thickness of contaminated soil, strong colour, marked odour), but no discrimination was made between a soil profile showing either a high or low contamination. In all, 41 sampling sites and 4 observation sites were selected along the riverbank, and included sites on 6 islands on the Saint-François River (Fig. 1). A Global Positioning System (GPS) survey was done at each site so that each one could be located on the mapping medium using the ArcGIS software program (versions 8.2 and 9.0). The sites were then positioned on orthophotographs at a scale of 1:40,000 (Fig. 1). This field work was used to delineate the riverbanks affected by the contamination, which extends from the municipality of Eustis (Massawippi River) to the municipality of Drummondville (Saint-François River). The sampling campaigns were done in late summer (August) or fall (September to October), when river water levels are usually lower.

Analysis of Soil Properties and Contaminated Layer

Of the 41 sites, 20 soil profiles along the riverbanks were selected for sampling the soil materials (horizons) and the contaminated layer. Another contaminated layer was also sampled near the former Eustis mine (EUS), where traces of hydrocarbons were visible on the surface. Samples of contaminated soils were also taken from a public beach (STO-1) at Drummondville (a total of 22 sites including EUS and STO-1 sites). For the 20 soil profiles, a trench was dug along the riverbank in order to reach the contaminated layers, which in some cases were found at a depth of more than one meter. The data collected in the field consisted of the depth at which the contaminated soils were found, the distance separating the trench from the riverbank, and the description of the soil profile (e.g., facies and soil texture, colour of horizons, stoniness), and the soil samples were collected for laboratory analysis. The morphological and properties analysis of the soil was done on the same profiles that were used for the contaminated layer sampling. For each profile, the collected soil horizons were identified in the field using the criteria of the Canadian System of Soil Classification (CSSC) (SCWG 1998) and the Canada Soil Information System (CanSIS 1982). The thickness of the soil horizons, their texture and colour (Munsell chart), and structure and stoniness were noted. The morphological analysis also helped to properly position the limits of the contaminated layers in each of the analyzed profiles. Soil samples were collected and analyzed in the laboratory to better characterize the texture of the deposits and evaluate chemical characteristics (pH, total organic carbon) based on the CSSC's criteria (SCWG 1998). For each soil profile, the samples (±500 grams) were collected at defined depths (e.g., 20, 40, 60, 80, 100 cm) and used for the physical and chemical analyses. All of the samples were air-dried and passed through a 2-mm stainless steel sieve. For the grain size analysis, the sandy fraction was obtained by sieving, while the finer fractions were obtained using a hydrometer. The methods used for the chemical analysis consisted of determining the pH in CaCl₂ (0.01 M) using a 1:2 soil to solution ratio (Carter 1993), and the total organic carbon (TOC) content using the method developed by Yeomans et al. (1988). For the contaminated layer, the sampling method consisted in taking samples of soils that were mixed in a receptacle to ensure the homogeneity of the sample. A total of 34 samples from 22 stations was used (two samples by station), including 22 samples for the analysis of hydrocarbons (C₁₀ to C₅₀) and 12 other samples for the analysis of other contaminants (heavy metals, PCBs, and PAHs). The soil samples from the contaminated layer were collected according to the depth of the layer, which varied considerably from one profile to another. No duplicates of soil materials were taken in the field. The soil samples were then stored in sterile containers (glass bottles) and refrigerated at the site. The samples were used to analyze C₁₀ to C₅₀ hydrocarbons, PCBs, PAHs, and heavy metals. The analysis for the hydrocarbon samples was done using gas chromatography coupled with a flame ionization detector (GC-FID). The detection limit for the C₁₀ to C₅₀ hydrocarbons was 60 mg/kg of dry matter. The results were indicated by a regression curve (data not shown) and expressed as mg/kg of petroleum hydrocarbons (C₁₀ to C₅₀), given the nature of the samples (dry soil materials). The analysis protocol was completed by an outside laboratory (Biolab Inc.) accredited by the Ministère du Développement durable, de l'Environnement et des Parcs du Québec (MDDEP 2007; see also http://www.ceaeq.gouv.qc.ca/methodes/chimie_org.htm#bpc_cong). The heavy-metal content was analyzed for the soil samples collected in the contaminated layer. These analyses were done according to procedures drawn up by the MDDEP (2007). The samples were dissolved using HNO₃. The resulting liquid residue was then analyzed using plasma mass spectrometry (ICP-MS). The complete procedures used by Biolab Laboratory are available in the government documents (CEAEQ 2005a), and the laboratory is certified by the Ministère de l'Environnement du Québec, which requires duplicate analyses to validate the laboratory tests (MEQ 2004). The quality control and acceptability criteria (QA/QC) had to conform to government document DR-12-SCA-01 (CEAEQ 2005b) and were applied as follows: (i) the curve determination coefficient should be at least 0.995, and (ii) the duplicate analytical method must be under the methodological
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Quantification limit. The randomly tested check standards should not exceed 20% of the target value, except for the level 1 standard, which is used more to ensure that sensitivity is adequate.

Results and Discussion

Spatial Distribution of the Contamination in the Riverbanks

The field work along the riverbanks of the Saint-François and Massawippi Rivers revealed that the contamination extends from the small municipality of Eustis to the municipality of Drummondville (Saint-Nicéphore sector) (Fig. 1). No other traces of contaminants were found outside the boundaries of the river section. From Eustis to Drummondville, both riverbanks are contaminated with hydrocarbons (C_{10} to C_{50}) over about 103.5 km. Contaminated soil was also found on the six islands between Windsor and Drummondville. The banks of the Massawippi River, starting from the former Eustis mine (MAS-13-2) sites), contain contaminants that extend over several kilometers downstream (MASO-1 MASO-2, MASO-13, and MASO-13-2 sites). However, no contamination was observed (no visual and olfactory traces) in the soil materials of the banks upstream of the Eustis mine site. If one considers just the thickness of the contaminated soil (not the concentration of hydrocarbons), the most problematic sites are those in the Massawippi sector (e.g., MAS-13 and MAS-13-2). The contaminated layers may extend over more than one meter in depth in the riverbank soil (Fig. 2), and the colouration and odour are more pronounced. However, the values obtained for the concentration of hydrocarbons in this sector do not exceed 300 mg/kg, except for the EUS site (380 mg/kg) where the samples were taken from the former mine site.

None of the documents consulted (e.g., government reports, archives, or newspapers) showed any contamination by hydrocarbons or petroleum products along the Saint-François and Massawippi Rivers. The only information obtained comes from two regional newspaper articles indexed by the Société d’histoire de Sherbrooke. Based on the information recorded in the newspapers, the pollution events in the Saint-François River seem to have occurred on two separate occasions, i.e., October 25, 1955 and May 17, 1963, though no details on the events or their causes are provided (La Tribune 1955, 1963). Furthermore, no mention in the articles is made of the pollution’s origin (e.g., discharge of oil spills or other contaminants). The October 1955 article mentions a case of pollution extending over 45 miles (±72 kilometres) along the Saint-François River, but this water pollution would be more associated with industrial waste, such as from pulp and paper mills. The May 1963 article mentions that the pollution was found within the municipality of Sherbrooke (around the Aylmer bridge in the downtown area). We read that, along the Saint-François River, there were large oil slicks whose source was unknown. However, it is probable that other contamination events (e.g., local contamination) occurred but were not mentioned in the documents that were consulted. Finally, the amount and nature of the hydrocarbons released into the rivers remains entirely unknown, but these pollutants must be sufficiently soluble to penetrate into the riverbank soil materials. These pollutants can also migrate into soil and thus spread through the vertical and lateral layers of the profile (Cozzarelli et al. 2001). However, a minimal amount of contaminant is required for such dissemination in the soil materials, and the properties of the fluid must be relatively immiscible (BRGM 2001).

Concentration of Contaminants

The C_{10} to C_{50} hydrocarbon concentrations varied from one site to the next. The samples taken from the Windsor sector (STO-4 and STE-11) and on Morin Island (ISL-4) show the highest levels of contamination (Table 1). In addition, various sites show moderate contamination (STO-14 and STO-15). For the soil samples for which the concentration of hydrocarbons indicated values between 300 and 700 mg/kg (B criterion), contaminated materials were considered. These levels create certain
usage constraints since the standards issued by the Ministère du Développement durable, de l’Environnement et des Parcs du Québec (MDDEP 2007; see also http://www.mddep.gouv.qc.ca/sol/terrains/politique/annexe_2_tableau_1.htm) state that any contaminated soil with contaminant levels ranging within the B criterion (B to C level) means that the soils contain organic or inorganic contaminants. According to the Policy on Soil Protection and the Rehabilitation of Contaminated Lands (MDDEP 2007), contaminated soil is categorized based on the following criteria: contamination levels A to B, residential uses; levels B to C, industrial uses; levels >C, use is prohibited without treatment. Upstream of Windsor, soil contamination in the riverbanks (STE-8 and STO-7) was less concentrated but still present (<80 mg/kg). The samples taken from the Richmond (ISL-4) and Windsor (STO-4 and STE-11) sectors (Fig. 1) showed a relatively high C10 to C50 hydrocarbon contamination rate, i.e., 582 and 660 mg/kg, respectively (Table 1). Lastly, the lowest concentration levels were found in the samples taken from the municipal beach in Drummondville (STO-1) and at the Massawippi site (MAS-13), which was contaminated at a hydrocarbon level of <60 mg/kg of dry matter. This concentration corresponds to the hydrocarbon detection limit and does not represent a potential hazard according to the MDDEP’s contaminated soil criteria (MDDEP 2007). Note that soil with less than 300 mg/kg of dry matter is part of the A criterion, which does not involve any usage constraints (Beaulieu 1998; MDDEP 2007).

With respect to heavy metals, PCBs, and PAHs, 12 samples were analyzed. These were the same soil profiles (contaminated layer samples) that were used for the C10 to C50 hydrocarbon analysis. The results obtained showed low levels of heavy metal, PCB, and PAH contamination, except for the STO-10, STO-13, STO-15, and STE-8 sites, which show higher concentrations for As, Cd, Cu, and Zn (Table 2), and the concentration levels of the samples (Cu and Zn) all fall under the B or C criterion. According to the consulted reports (Berryman 1996; Berryman et al. 2003; Cogesaf 2006; Painchaud 2007), these toxic metals and other contaminants (e.g., PCBs) were detected in benthic fauna and fish found in the Saint-François River. For instance, in the flesh of white sucker (Catostomus commersoni), high concentrations of PCBs in the order of 1,500 to 1,700 μg/kg were detected (Painchaud 2007); these very high values are close to the levels considered to be harmful (≥2,000 μg/kg) according to the criteria established for the protection of bird and mammal predators (MDDEP 2006). Also, 17% of the fish collected in the Windsor-Richmond area, including northern pike (Esox lucius), showed abnormalities (e.g., tumours).

Based on the results obtained, it seems difficult to account for the differences noted in the heavy metal and hydrocarbon levels at the various sites. One could expect to find the highest concentration levels around the Eustis...
Contaminated Soils in Riverbanks (Southern Québec)

mine (MAS-13 and MAS-13-2), the probable location of the discharge, whereas the highest concentrations for hydrocarbons (C_{10} to C_{50}) were actually found in the Windsor and Richmond sites (STO-4, STE-11, and ISL-4). The lowest levels of contamination in the Massawippi River soil samples could possibly be explained by a higher dissolution of pollutants through fluctuations in the water table, thus resulting in a faster dispersion of contaminants by leaching. The Massawippi River is prone to periodic flooding (Jones 1998; Saint-Laurent et al. 2001; Saint-Laurent and Saucet 2003), which causes the banks to overflow on numerous occasions during heavy rain and frequent variations in the river water level. It is therefore likely that these variations in river water level would create frequent variations in the water table (Krause and Bronstert 2007), and in so doing, would lead to rapid leaching of the contaminants. A similar observation was made by Ciszewski and Malik (2004) in their study of the Mala Panew River in Poland, which showed a major decrease in the concentration of heavy metals in the soil in less than 40 years due to frequent variations in the water table. In our case, a more in-depth study would be needed using piezometers along the banks to truly assess this hypothesis on the effects of fluctuating water levels. Lastly, there have likely been other spills (local contamination) in the Windsor and Richmond areas, which could explain why higher contamination levels are seen along these banks. However, a document search (e.g., government reports, newspapers, archives) did not reveal any information on the above events.

One could wonder also whether the differences detected in the concentrations of contaminants could be explained by pH, textural variations, and/or the organic carbon content in the riverbank soil materials. Various
studies showed that the presence of organic matter in the soil favours greater water retention and thus contributes to retaining hydrocarbons and heavy metals in the sediment (Calvet 1989; Bubb and Lester 1996; Hayden et al. 1997). The acidity of the soil or sediment can also increase the mobility of heavy metals (Calvet 1989). It is also known that contaminant levels usually decrease in soil textures ranging from fine to coarse (e.g., clay loam, silt, loamy sand, fine sand) and based on depth (de Groot et al. 1982; Bubb and Lester 1996; Nikanorov and Stradomskaia 2003). In other words, one can expect to find higher contaminant levels in sediment with a fine texture and high organic content.

Some physical and chemical properties (e.g., pH, TOC) of the soil samples collected in the contaminated profiles are presented in Fig. 3, 4, and 5 and in Table 2. The soils that were analyzed generally showed relatively low pH values (Fig. 3), ranging from 3.56 to 7.09 with a median value of 5.45 (Table 3). In some cases, acid samples were generally found at the base of the soil profile (STO-12 and STO-14). TOC ranged from 0.06 to 1.56%, with an average value of 0.62% and a median value of 0.63 (Table 3). The TOC concentrations are generally low values in the soil profiles (Fig. 4), with a few exceptions (STO-12 and STO-16). A slightly higher organic matter concentration was usually observed in the subsurface of the solum, but this was not the case for all the soil profiles (e.g., STE-8, STE-11 and STO-15). The data obtained from the soil texture classes examined are

### Table 3. Means, medians, standard deviations (SD), and ranges for the properties in the soil samples

<table>
<thead>
<tr>
<th></th>
<th>pH (CaCl₂)</th>
<th>TOC</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.36</td>
<td>0.62</td>
<td>61.8</td>
<td>28.2</td>
<td>10.1</td>
</tr>
<tr>
<td>Median</td>
<td>5.45</td>
<td>0.63</td>
<td>63.6</td>
<td>27.2</td>
<td>10.3</td>
</tr>
<tr>
<td>SD</td>
<td>0.89</td>
<td>0.35</td>
<td>14.1</td>
<td>12.6</td>
<td>11.2</td>
</tr>
<tr>
<td>Range</td>
<td>3.56 – 7.09</td>
<td>0.06 – 1.56</td>
<td>35.6 – 94.3</td>
<td>3.3 – 52.1</td>
<td>2.4 – 25.1</td>
</tr>
</tbody>
</table>
relatively similar from one site to the next (Fig. 5). The dominant textures ranged from very fine sand to fine and very fine sandy silt. The proportion of sand in the soil samples ranged from 35.6 to 94.3%, with a mean value of 61.8%, and the proportion of clay ranged from 2.4 to 25.1%, with a mean value of 10.1% (Table 3).

Additional data on pH, soil texture, and TOC concentration levels in riverbank sediments from neighbouring sites showed relatively comparable results (Lavoie et al. 2006), i.e., little variation in pH and texture and fairly low organic matter content in the soil surface (±1-meter depth). In short, there was not much variation in pH, soil textural class, and the respective TOC concentrations from one site to the next. For instance, in the soil profiles with low or higher contaminant levels (hydrocarbons C_{10} to C_{50}), the texture, pH, and TOC values were relatively comparable (Fig. 3, 4, 5). This leads us to believe that these parameters do not account for the differences observed in the hydrocarbon concentrations between the sites analyzed. In this regard, the study by Martin (2004) conducted in the Lahn River area (Germany) reached similar conclusions, namely that there was a low correlation between texture and organic matter content and the heavy-metal levels detected in terrace sediments. Other studies (Taylor 1996; Leece and Pavlowsky 1997) reached appreciably the same conclusions as Martin (2004), mentioning a weak relationship between metal concentrations and fine-textured, organic-rich sediments.

**Conclusion**

This study revealed a recent case of spatially delineated contamination along the banks of the Saint-François and Massawippi Rivers, and identified the type and concentration of the contaminants found in the riverbank soils. The contamination most likely originated from discharges at a former mining site (Eustis mine). The riverbank contamination extended for over 100 kilometres between the municipalities of Eustis and Drummondville, and the contaminants primarily consisted of hydrocarbons (C_{10} to C_{50}), heavy metals, PCBs, and PAHs. The pollutants (heavy metals, PCBs, and PAHs) indicated low levels of contamination except for some metals (Cu and Zn). For hydrocarbons, the highest levels of contamination were found at the Morin Island and Windsor sectors (ISL-4, STE-11, and STO-4), and at moderate levels for some other sites (EUS, STO-10, STO-14 and STO-15); the levels ranged from 300 to 700 mg/kg and are part of the B criterion. This level creates certain usage constraints since the standards are issued by the Ministère du Développement Durable, de l’Environnement et des Parcs du Québec (MDDEP 2007). Considering the soil properties, the texture, pH, and TOC values were relatively comparable, and the same was true for the profiles with low or higher contaminant levels. This analysis also showed that although the polluting discharge was produced many years before, traces are still found today. Finally, it is to be hoped that government policies will be more stringent toward polluting industries in order to ensure a higher level of environmental protection.

**Acknowledgments**

The authors would like to thank all the individuals and organizations that contributed to this study, including the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Fondation institutiel du recherche (FIR-UQTR) for their financial support. We would like to also acknowledge Luc Lavoie and François Pelcouni for their valuable assistance during field work.

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Received: 29 May 2007; accepted: 16 June 2008.
Distinguishing the Hydrochemistry of Two Hydrological Basins in Northern Mexico Using Factor Analysis

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Hydrochemical parameters of groundwater from two hydrological basins in northwestern Mexico were measured. In one of them is located the city of Puerto Peñasco, and in the other one is the city of El Rosarito. A factor analysis was used to characterize the main influences that affected the water quality of each region. Based on the results of this method, the aquifer located in Rosarito is mainly affected by seawater intrusion and the presence of high levels of manganese, while the groundwater characteristics at Puerto Peñasco are influenced by reductive conditions, probably caused by bacterial contamination. Although most of the parameters analyzed in this study were within normal ranges for groundwater, knowledge of the factors affecting sources of water can help to develop restoration projects and preventive management practices to prevent an irreversible degradation of groundwater quality.

Key words: groundwater, factor analysis, northwestern Mexico, nutrients, trace metals

Introduction

Mexico is one of the largest users of groundwater in the world and faces growing challenges for the management of water resources (Scott and Shah 2004). Ninety percent of the country’s irrigation zones and 70% of its industrial plants are located in the northern region, which receives less than 40% of the country’s total rainfall (Sandoval 2003). Droughts in northern Mexico in recent years have exacerbated water deficits. Mexico’s National Water Commission along with other federal and state water management institutions have made significant efforts to respond to the country’s growing water demands (Hearne 2004; Sandoval 2004). But despite regulatory measures to reduce groundwater overdraft, in many regions, pumping continues to exceed aquifer recharge and leads to declining water tables and deterioration of groundwater quality (Scott and Shah 2004).

Groundwater quality is influenced by several factors, including evapotranspiration, salinization, and rock-water and soil-water interactions (Liu et al. 2003). Hydrochemical analyses have been used throughout Mexico in recent years to assess groundwater quality and contamination by wastewater (Barragan et al. 2001; Dominguez-Mariani et al. 2004; Muñoz et al. 2004). However, available water quality records from northern Mexico are scarce. Factor analysis is an integral statistical method that can help to define and evaluate the structural-functional organization of systems (Kaplunovsky 2005). Factor analysis techniques have been applied to aquifers to achieve a variety of objectives such as identifying chemical and physical groups for the delineation of optimal operational zones (Melloul 1995), comparing groundwater composition of different survey areas (Helena et al. 2000), identifying salinization attributed to sea water intrusion (Morell et al. 1996), tracing ground water circulation in volcanic terrains (Join et al. 1997), and assessing groundwater contamination by anthropogenic activities (Subbarao et al. 1996). The present study used factor analysis to compare the water quality from two hydrological basins in northwestern Mexico. In one of them is located the city of Puerto Peñasco, while in the other one is located the city of El Rosarito. The two areas have very different rainfall patterns even though they are at the same latitude and within a distance of a few hundreds of kilometers. Under that condition we wanted to establish the degree to which weather and anthropogenic activities could affect groundwater quality in these areas. Puerto Peñasco has an average annual rainfall of 109 mm, occurring mostly in the summer, and is considered one of the most arid regions of México. El Rosarito has an average of 175 mm, occurring mostly in winter with a Mediterranean weather pattern (CNA 1991); this means that the territory has a distinctive wintertime precipitation regime (Pavia and Graef 2002).

Materials and Methods

Study Area

Puerto Peñasco is located in northwestern Sonora, south of the Altar Desert. The groundwater was sampled from
The area is characterized by hot, dry conditions throughout most of the year, with long episodes of low precipitation in which the water supply becomes very low. In the last 70 years, three critical low rainfall periods have occurred: the 1930s, 1950s, and 1990s (Brito-Castillo et al. 2003). Puerto Peñasco is located in the basin of the Altar and Bamori rivers, within the irrigation districts of Altar-Pitiquito-Caborca and part of the Colorado River. Since surface water is scarce, both districts rely on groundwater from the aquifer. Well water is used for agriculture, cattle ranching, food processing (fish processing), and domestic activities (Cervantes-Rosas and Arredondo-Garcia 1999). Puerto Peñasco has been growing rapidly, and it currently occupies 977,443 hectares with a population of 31,200 (INEGI 2000b). Some wells in the area surpass 100 m in depth (Table 1), indicating that the static level of the aquifer is very deep (INEGI 1981b). Fishery and tourism are the main economic activities in Puerto Peñasco.

El Rosarito is on the west coast of the Baja California Peninsula. Groundwater was sampled from 9 wells (Fig. 1). The study area is located in a semi-arid region south of Tijuana, inside the Tijuana-Arroyo de Manedero river basin (INEGI 1981a). The average annual precipitation increases from 175 mm near the coast to 350 mm in the Juarez Mountains to the east (CNA 1991). This area has a high population density of 63,400 within 3,400 hectares (INEGI 2000a). The main uses of water are residential and commercial (INEGI 2000a).

![Fig. 1. Location of stations in the hydrological basins in Rosarito, Baja California, and Puerto Peñasco, Sonora.](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Puerto Peñasco, Sonora</th>
<th>Rosarito, B.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth</td>
<td>m</td>
<td>Min. 79</td>
<td>Max. 150</td>
</tr>
<tr>
<td>TDS</td>
<td>mg/L</td>
<td>Min. 916</td>
<td>Max. 1612</td>
</tr>
<tr>
<td>pH</td>
<td>—</td>
<td>Min. 8.18</td>
<td>Max. 10.4</td>
</tr>
<tr>
<td>E.C.</td>
<td>µS/cm</td>
<td>Min. 1652</td>
<td>Max. 2873</td>
</tr>
<tr>
<td>Hardness</td>
<td>mg/L</td>
<td>Min. 63</td>
<td>Max. 118</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>mg/L</td>
<td>Min. 192</td>
<td>Max. 394</td>
</tr>
<tr>
<td>Ca</td>
<td>mg/L</td>
<td>Min. 8.89</td>
<td>Max. 23</td>
</tr>
<tr>
<td>Mg</td>
<td>mg/L</td>
<td>Min. 9.91</td>
<td>Max. 21.84</td>
</tr>
<tr>
<td>Na</td>
<td>mg/L</td>
<td>Min. 221</td>
<td>Max. 395</td>
</tr>
<tr>
<td>Mn</td>
<td>mg/L</td>
<td>Min. 0.013</td>
<td>Max. 0.032</td>
</tr>
<tr>
<td>Cu</td>
<td>mg/L</td>
<td>Min. 0.005</td>
<td>Max. 0.007</td>
</tr>
<tr>
<td>Fe</td>
<td>mg/L</td>
<td>Min. 0.068</td>
<td>Max. 0.894</td>
</tr>
<tr>
<td>Cl</td>
<td>mg/L</td>
<td>Min. 562</td>
<td>Max. 998</td>
</tr>
<tr>
<td>N-NH₄</td>
<td>mg/L</td>
<td>Min. 0.011</td>
<td>Max. 0.146</td>
</tr>
<tr>
<td>N-NO₂</td>
<td>mg/L</td>
<td>Min. 0.009</td>
<td>Max. 0.05</td>
</tr>
<tr>
<td>N-NO₃</td>
<td>mg/L</td>
<td>Min. 0.97</td>
<td>Max. 1.4</td>
</tr>
<tr>
<td>P-PO₄</td>
<td>mg/L</td>
<td>Min. 0.014</td>
<td>Max. 0.168</td>
</tr>
</tbody>
</table>

*E.C. = electrical conductivity.

 bà.n.d. = not detected.
Sample Collection and Analysis

Water samples from 26 wells were analyzed. In both areas the distribution of the sampled wells was representative of the extent of each hydrological basin. The locations of the wells were determined with a portable GPS (Magellan 315, Thales Navigation, Santa Clara, Calif.). Preservation of samples and analytical protocols were conducted according to standard methods for surface water analyses (APHA 1992). Samples from the wells were taken in bottles free of phosphates and heavy metals. A total of 16 variables were measured in each sample. In the field, conductance and temperature were determined with a salinometer (YSI-85), and pH was determined with a pH meter (Orion 290A). In the laboratory, calcium (Ca), magnesium (Mg), sodium (Na), copper (Cu), iron (Fe), and manganese (Mn) were analyzed using an atomic absorption spectrophotometer (GBC model AVANTA). Analytical data were verified with standard, blank measurements, duplicate samples, and spikes after every block of ten samples. Ammonium (N-NH₃), nitrites (N-NO₂), nitrates (N-NO₃), and orthophosphates (P-PO₄) were analyzed with an auto-analyzer of ions with a continuous flow FIAS (Latchat model Quik Chem 8000). Chlorides (Cl⁻) and sulfates (SO₄²⁻) were analyzed using a spectrophotometer (Spectronic 21D) according to the methodologies recommended by APHA (1992).

Statistical Analyses

Hydrochemical data were statistically analyzed by factor analysis using a varimax-rotated empirical orthogonal function. To reduce the number of variables and detect structure in the relationships between variables, factor analytic techniques were used to classify variables. Series of data of two or more variables that are in a scatter plot generate a regression line that represents the “best” summary of the linear relationship between the variables. Therefore, two or more variables were reduced in a linear combination called a factor. During the computational process, the Cartesian axes were rotated. The objective was to maximize the variance (variability) of the factor while the variance around the factor was minimized. This type of rotation is called variance maximizing. After the first line had been drawn through the data, we continued and defined another line that maximized the remaining variability. This process continued until the totality of the variance was covered. During this process, consecutive factors were extracted. Because each consecutive factor was defined to maximize the variability not considered by the preceding factor, consecutive factors were uncorrelated or orthogonal, with a minimum independence between each other (Hill and Lewicki 2007).

The series of data were also analyzed by calculating the correlation index with the objective of evaluating the relationship between the variables. Both statistical methods were done using STATISTICA computer software, version 5.0. Factor analysis and correlation index offer a more complete understanding of water quality and the status of groundwater systems than traditional quantitative methods. Both statistical methods determine the relationships between chemical variables and identify possible sources and factors that influence water systems (Liu et al. 2003; Simeonov et al. 2003).

Results and Discussion

Water Quality

The mean, minimum, and maximum levels of the hydrochemical analyses are shown in Table 1. Although 71 underground water sources have been recorded in the basin in which Puerto Peñasco is located (SIUE 1999), of those, only 17 were operating in 2003. The wells sampled in this study were only domestic wells.

The static levels of the Puerto Peñasco wells ranged from 3.21 to more than 150 meters below ground surface (Table 1). Water level is mainly affected by the extraction through wells. The increase of ground water is very limited by the scarce rain precipitation in this region or other sources of refilling. In some coastal areas, water filtration occurs from the aquifer to the sea (SIUE 1999). The positive migration of water from the ground aquifer to the sea has reduced seawater intrusion into the aquifer (SIUE 1999). However, evidence of seawater intrusion was recorded in some of the areas around Puerto Peñasco. On the other hand, in El Rosarito, static levels ranged from 3.47 to 12.80 metres below ground surface. The monitored wells in this area are mainly affected by domestic extraction.

The total dissolved solids (TDS) in the groundwater of Puerto Peñasco area ranged from 115 to 1,612 mg/L, with a mean of 916 mg/L, and is classified as oligohaline (Por 1972), in contrast to the values for El Rosario, which are much higher with a mean of 2,448 mg/L and a range from 763 to 5,157 mg/L that is almost three times the average concentration in Puerto Peñasco. The high levels of TDS in both aquifers suggest seawater intrusion, which is corroborated by a high average of chloride concentrations (562 and 1,516 mg/L respectively) greater than 250 mg/L, the maximum level recommended for human consumption (WHO 1996). The chloride levels in El Rosarito are almost three times higher than in Puerto Peñasco. The combination of high levels of TDS and chloride was the reason of the discontinued use as a water supply from the El Rosarito aquifer in 2001. Therefore, the future use of this aquifer seems to be very difficult because chloride is a conservative ion that is difficult to remove by most processes other than precipitation at very late stages of salinization (Richter and Kreitler 1993).

The pH level was found outside of the acceptable range (pH 6.5 to 9.5 [WHO 1996]) in only one sample from Puerto Peñasco (Well 12, pH 10.4). Well 12 also had Fe concentrations (0.894 mg/L) above the recommended level for drinking water (0.30 mg/L [WHO 1996]). The Mn and Cu concentrations in all wells of Puerto Peñasco...
Ammonia does not generally have direct relevance to the maximum tolerable limit (0.2 mg/L [WHO 1996]). In one sample (0.87 mg/L from Rosarito Well 12) over the maximum tolerable limit (0.2 mg/L [WHO 1996]). Ammonia concentration was found only in one sample (0.87 mg/L from Rosarito Well 12) over the maximum tolerable limit (0.2 mg/L [WHO 1996]). Ammonia does not generally have direct relevance for human health, but it is an important indicator of fecal contamination and disinfection efficiency, and its presence in high levels causes unfavorable taste and odor (WHO 1996). Phosphates are not included in the list of drinking water contaminants (Environmental Protection Agency 2002). However, higher concentrations could be indicative of agricultural activities, domestic pollution, or extensive degradation of subterranean organic matter (Meybeck 1982; Griffioen 2006). Natural dissolved inorganic phosphate in river water should average about 0.01 mg/L, which is a similar level to that found in both zones. Phosphate concentrations up to 14 mg/L are related to waste disposal (WHO 1996).

Aquifer Characterization

Based on the chemical profiles shown in Tables 2 and 3, the two aquifers have different composition patterns. Five principal components with eigenvalues >1 in Puerto Peñasco and Rosarito explained 92 and 90%, respectively, of the total variance in the water quality data set (Table 2). For loading values of the variables greater than 0.75, 0.75 to 0.5, and 0.5 to 0.3, the designations “strong,” “moderate,” and “weak,” respectively, are used (Liu et al. 2003).

In Puerto Peñasco, the first factor explained 48% of the total variance with strong negative factor loadings for pH, Fe, NO3, and PO4 (Table 3, A). These parameters are associated with reductive environments characterized by high levels of soluble iron and nitrates. The second factor explained 19% of the total variance with a strong positive factor loading for TDS, conductivity, and chlorides, and a strong negative factor loading for ammonium, indicating sewage or agricultural contamination (Kampbell et al. 2003; Panno et al. 2006). Bacteriological pollution was not found in municipal drinking water from the city of Nogales, Sonora, although, up to 160 million counts per 100 mL of total coliforms were recorded in samples from wells used to supply water to areas not covered by the municipal system (Sanchez 1995).

The third factor explained 12% of the variance with strong positive factor loadings for Cu, Mg, and hardness, indicating leaching from overlying soils probably caused by water extraction, which results in an increase of minerals in the water. The fourth factor explained 7% of the total variance with a strong negative loading for alkalinity and a weak loading for nitrates; both values indicated a biological denitrification process that results in decreasing levels of alkalinity and nitrates (Flores III et al. 2007). The fifth factor explained 6% of the variance with a strong negative loading for Ca and a moderate positive loading for Na, indicating influences of seawater intrusion into the aquifer.

In El Rosarito (Table 3, B), the first factor accounted for 43% of the total variance with strong positive loadings in TDS, conductivity, Cu, Ca, hardness, and chlorides, indicating major salinization of the aquifer from seawater intrusion, typically a result of water extraction (Simeonov...
Aquifer Characterization Using Factor Analysis

et al. 2003), confirming our observation previously stated in this manuscript at the beginning of the discussion. The second factor accounted for 18% of the total variance with a strong positive loading for pH and a strong negative loading for nitrates, perhaps indicative of organic contamination problems. The third factor accounted for 13% of the total variance with a strong negative loading for alkalinity, nitrates, and phosphates, which can also be related to a biological denitrification process (Flores III et al. 2007), such as in Puerto Peñasco, and a precipitation of phosphates. The fourth factor accounted for 9% of the total variance with strong positive loadings for Mn and Fe, indicating reduced conditions. This conclusion was corroborated by the strong odor of H2S detected in some of the wells. The fifth factor explained 7% of the total variance with a moderate loading for ammonium.

Relationships Between Water Components

In both areas, chloride is the anion present in the highest concentrations (Table 1). Chloride in Puerto Peñasco (Table 4) is associated with nitrites, nitrates, ammonium, and phosphates, probably indicating sewage influence (DeSimone and Howes 1998) and redox reactions. Phosphate concentrations associated with ammonium and CO2 pressure in near-neutral pH pressure are related to low levels of oxygen and organic matter degradation (Griffioen 2006). Chloride in Rosarito is associated with hardness (Table 5), confirming that it is a noncarbonate hardness type.

In Rosarito (Table 5), Na significantly correlates with hardness ($r = 0.73$, $p < 0.001$), while in Puerto Peñasco (Table 4), it correlates with alkalinity ($r = 0.51$, $p < 0.04$).

| Table 2. Eigenvalues, percentage of variance, cumulative eigenvalues, and cumulative percentage of variance for the varimax-rotated factor analysis of hydrochemical constituents of ground water at Puerto Peñasco, Sonora (A) and Rosarito, B.C., México |
|-----------------|-------------|-------------|-------------|-------------|
|                | Eigenval.   | % total     | Cumul.      | Cumul.      |
|                | variance    | Eigenv.     | variance    | %          |
| A. Puerto Peñasco |
| 1               | 7.6874      | 48.0465     | 7.6874      | 48.0465     |
| 2               | 2.9916      | 18.6974     | 10.679      | 66.7348     |
| 3               | 1.9571      | 12.2316     | 12.6361     | 78.9755     |
| 4               | 1.0717      | 6.6981      | 13.7078     | 85.6735     |
| 5               | 1.0307      | 6.442       | 14.7385     | 92.1156     |
| 6               | 0.4225      | 2.6405      | 15.161      | 94.7561     |
| 7               | 0.336       | 2.0999      | 15.497      | 96.856      |
| 8               | 0.2172      | 1.3575      | 15.7142     | 98.2135     |
| 9               | 0.1331      | 0.8318      | 15.8472     | 99.0453     |
| 10              | 0.0762      | 0.4763      | 15.9235     | 99.5216     |

B. Rosarito

|                | Eigenval.   | % total     | Cumul.      | Cumul.      |
|                | variance    | Eigenv.     | variance    | %          |
| 1               | 6.8818      | 43.0111     | 6.8818      | 43.0111     |
| 2               | 2.8092      | 17.5577     | 9.691       | 60.5688     |
| 3               | 2.0601      | 12.8757     | 11.7511     | 73.4445     |
| 4               | 1.4847      | 9.2796      | 13.2359     | 82.7241     |
| 5               | 1.1903      | 7.4395      | 14.4262     | 90.1637     |
| 6               | 0.6516      | 4.0728      | 15.0778     | 94.2365     |
| 7               | 0.3012      | 1.8825      | 15.379      | 96.119      |
| 8               | 0.24        | 1.5         | 15.619      | 97.619      |
| 9               | 0.0476      | 0.2976      | 15.6667     | 97.9167     |
| 10              | 0.0476      | 0.2976      | 15.7143     | 98.2143     |

| Table 3. Loading factors obtained in the factorial analysis for Puerto Peñasco, Sonora (A) and Rosarito, B.C., México |
|-----------------|-------------|-------------|-------------|
|                | Factor 1    | Factor 2    | Factor 3    | Factor 4    | Factor 5    |
| TDS             | 0.3505      | 0.8520      | -0.1202     | 0.2538      |
| pH              | -0.9262     | -0.2936     | -0.1087     | 0.2147      | -0.1127     |
| COND            | 0.3653      | 0.8513      | 0.2315      | -0.1243     | 0.2403      |
| Mn              | -0.5933     | -0.0969     | -0.1265     | 0.2233      | -0.6096     |
| Cu              | -0.1090     | 0.0715      | 0.3528      | -0.2857     | 0.1596      |
| Fe              | -0.9573     | -0.1258     | -0.2164     | 0.0956      | 0.0139      |
| Ca              | 0.1683      | -0.4701     | 0.2875      | -0.1526     | -0.7612     |
| Mg              | 0.3374      | 0.3386      | 0.7805      | 0.3187      | 0.1320      |
| Na              | 0.2598      | 0.3006      | 0.2335      | -0.3843     | 0.7315      |
| Hard.           | 0.3818      | -0.0002     | 0.8225      | 0.1732      | -0.3289     |
| Alk             | 0.2901      | 0.2261      | 0.0041      | -0.8217     | 0.0763      |
| N-NO3           | 0.8948      | 0.0847      | -0.1134     | 0.1719      | -0.1722     |
| N-NH4           | -0.3758     | 0.5082      | -0.0708     | -0.6449     | 0.2530      |
| Cl              | 0.2301      | -0.8367     | 0.1550      | 0.3278      | -0.0845     |
| Exp. Var.       | -0.9479     | -0.1409     | -0.0848     | 0.1579      | -0.0952     |
| Prp. Tot.       | 0.3855      | 0.8567      | 0.2185      | -0.0999     | 0.2060      |

**Note:** COND = conductivity; Hard. = hardness; Exp. Var. = explained variance; Prp. Totl. = proportion of total variance.
indicating that in Rosarito, Na is not associated mainly with the carbonate fraction. Therefore, in Rosarito, phosphates positively correlate with nitrates \( (r = 0.59, \ p < 0.01, \text{Table 5}) \), while in Puerto Peñasco, phosphates negatively correlate with nitrates \( (r = -0.53, \ p < 0.03, \text{Table 4}) \) and positively correlate with nitrates \( (r = 0.93, \ p < 0.001, \text{Table 4}) \). Both compounds, natural constituents, are at low levels, but the sites with high concentrations could be related to sewage or to the agricultural use of fertilizers or pesticides. In Puerto Peñasco, phosphates show a strong correlation with Fe \( (r = 0.98, \ p < 0.001, \text{Table 4}) \). During anoxic conditions, iron is reduced and suspended into the water column. When an oxygenation reaction occurs, iron is precipitated, again binding to phosphates (Griffioen 2006). This correlation could also indicate the presence of minerals such as strengite \( (\text{FePO}_4 \cdot 2\text{H}_2\text{O}) \) and metastrengite (Speiser and Kistler 2002) in this area. The strong correlation between nitrates and chlorides \( (r = 0.68, \ p < 0.001, \text{Table 4}) \) at Puerto Peñasco is a confirmative influence of domestic sewage on the water quality of the wells of this area.

**Conclusions**

Factorial analysis has been shown to be an adequate methodology for the detection of the possible causes that are affecting the quality of a hydrological basin. This statistical method gave several factors, however, the first two were enough to evaluate the main problems that affected the water quality of a hydrological basin, and they could be corroborated by using a correlation analysis which gives a clear relationship between the parameters evaluated.

Groundwater from areas near El Rosarito is mainly affected by seawater intrusion and the presence of high levels of manganese, while groundwater characteristics at Puerto Peñasco are influenced by reductive conditions that were probably caused by bacterial contamination, mainly by septic systems and domestic sewage. Puerto Peñasco is a very arid zone where the only source of fresh water is the aquifer. At Rosarito, the aquifer is no longer exploited officially but continues to be used locally. The evaluation of the degree in which environmental and

### TABLE 4. Matrix correlation for hydrochemical data in Puerto Peñasco, Sonora *

<table>
<thead>
<tr>
<th></th>
<th>TDS</th>
<th>pH</th>
<th>E.C.</th>
<th>Mn</th>
<th>Cu</th>
<th>Fe</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>Hard.</th>
<th>Alk.</th>
<th>N-NO₃</th>
<th>N-N₂O₅</th>
<th>N-NH₃</th>
<th>P-PO₄</th>
<th>Cl</th>
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<td>TDS</td>
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<tr>
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<td>0.01</td>
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</tr>
<tr>
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<td>0.60</td>
<td>-0.53</td>
<td>1.00</td>
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<tr>
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<tr>
<td>Fe</td>
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<td>0.59</td>
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<tr>
<td>Ca</td>
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<td>0.30</td>
<td>0.08</td>
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<tr>
<td>Mg</td>
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<td>-0.04</td>
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<td>0.51</td>
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<td>0.90</td>
<td>-0.47</td>
<td>0.65</td>
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<td>0.89</td>
<td>-0.03</td>
<td>-0.36</td>
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<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.06</td>
<td>0.02</td>
<td>0.00</td>
<td>0.98</td>
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<tr>
<td>N-NH₃</td>
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<td>-0.59</td>
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<td>-0.43</td>
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<td>1.00</td>
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<tr>
<td>P-PO₄</td>
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<td>0.01</td>
<td>0.93</td>
<td>0.75</td>
<td>0.70</td>
<td>0.06</td>
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<td>0.68</td>
<td>-0.60</td>
<td>-0.53</td>
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</tr>
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</table>

*Statistical significance \( (p) \) is indicated below each parameter.

\( ^b \ E.C. = \) electrical conductivity, \( ^b \) Hard. = hardness, \( ^b \) Alk. = alkalinity.
human impacts affect the quality of the water used for the population can help national and state water managers to develop more effective allocation practices to prevent irreversible degradation of the resource.

Acknowledgments

We thank the financial support provided by The Secretariat of Environment and Natural Resources (SEMARNAT), project 739-0 and CIBNOR projects PC 2.2 and PC 3.6. The staff editor at CIBNOR improved the English text.

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Received: 24 May 2007; accepted: 30 April 2008.
Potato Land Use and Nitrates Runoff Characteristics of Two Subcatchments of the Wilmot River Watershed, Prince Edward Island (PEI), Canada

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3University of Prince Edward Island, Charlottetown, PE Canada

Two subcatchments in the upper reaches of an arable watershed in central Prince Edward Island (PEI), farmed mostly to potatoes (in rotation), were monitored year-round for nitrate runoff. Land management inventories were done every fall and spring and assessed against nitrate runoff through regression analysis for the period 1991 to 2004. Nitrate concentration (averaging approximately 7 mg·L−1 over 14 years) in the outflow varied considerably yearly (standard deviation: 2.76) and monthly (standard deviation: 3.43), and exceeded quality guidelines (of 13 mg·L−1) for aquatic life in freshwaters at about a 6% frequency, averaging a nitrate-dollar loss of $1.70 per hectare per year.

Key words: nitrate runoff, water quality, sustainable agriculture, potato rotation, soil erosion

Introduction

The Wilmot River, one of several arable watersheds in Prince Edward Island (PEI), is situated just about centrally (Fig. 1) and occupies 81.2 km², much of which is intensively farmed mostly to potatoes (Solanum tuberosum) at commercial scales. It is PEI’s most important potato-producing basin, where the crop is grown in rotation with cereal grain and mixed forages, or with cereal grain alone.

The fields are heavily fertilized when in potatoes. Thus, with the focus on early growth and maximum yields, there is heavy reliance on inorganic nitrogen (N) forms, particularly nitrate (NO₃⁻). Therefore, based on its land usage, this watershed has been the target of much public criticism on suspicion of NO₃ contamination in the associated stream.

In global terms, the movement of surplus N from any farmland to watercourses (or subsurface sinks) is of concern to users of the affected resource where the element becomes a contaminant, usually in NO₃ form. Nitrate is widely recognized as a contributor to eutrophication and the resulting deleterious effects on fish and shellfish populations in estuaries (Sharpley et al. 1998). Thus, the copious algal growth that characteristically transforms PEI estuaries and bays, particularly Bedeque Bay, into ‘seas of green’ (to the point of public anxiety and media attention) strongly suggests NO₃ activity. There is little contest, therefore, with the notion that nitrate should be kept on the land where it was originally applied, at some cost, to nourish the intended crop. Hence, appropriate conservation management is meant to (a) achieve an economy of soil NO₃ within cropping systems, and (b) minimize water resource contamination.

Reducing NO₃ losses from farmland is undoubtedly a unifying concern for health professionals, environmentalists, organic farmers, and much of the consuming public who constantly exert pressure with an intent on (a) raising the consciousness of traditional producers and (b) influencing policy change in governments towards sustainable practices (Geier 1999) at all scales of production. Some governments, ranging from international to provincial (e.g., Sweden [Ulen 1997] and PEI [Royal Gazette 2002]) have even stipulated, in law, the adoption of “beneficial management practices” to conserve soil and minimize runoff and concomitant nutrient entrainment. These practices include: (a) cover cropping to reduce soil erosion (Edwards and Burney 1991) or to use up residual N (Jackson et al. 1993; Francis et al. 1998; Staver and Brinsfield 1998), (b) leguminous cropping (Wagger et al. 1998) and green manuring (Ambus and Jensen 1997) to reduce reliance on inorganic-N fertilizers, and (c) buffer zones to attempt to restrict overland flow into watercourses (Landry and Thurow 1997; Kronvang et al. 2000).

With the tendency towards ‘green’ legislation and increased farmer receptivity to sustainable agricultural precepts, policy makers, land use planners, and farmers alike will need measurements and a clear understanding of NO₃ mobilization from farmlands in order to advance policies and practices to minimize stream contamination. Hence, the present study is meant to build this understanding.

The objective of this study was therefore to assess the temporal distribution of NO₃ in the outflow from two commercially farmed subcatchments, and analyze
the relation of stream-water NO₃ to landuse and management methods in PEI's most agriculturally active watershed.

Materials and Methods

Location and Monitoring Facilities

The Wilmot River, situated near the centre of the province, flows westward from its upper reaches over a distance of about 18 km and empties into the Northumberland Strait at Bedeque Bay (Fig. 1) via an estuary situated about 5 km east of Summerside. The source tributaries of the river straddle the boundary between Queens and Prince Counties. The watershed, 81.2 km² in area, is about 80% farmed. The highest land elevation is 90 m above mean sea level in the upper reaches.

For the present study, two intensively farmed subcatchments along the steeper eastern boundary of this watershed were selected (identified in Fig. 2) for year-round monitoring of discharge (Burney 1989a, 1989b).

Topography, Climate, and Soils

The topography is undulating, and slopes reach upwards of 15% over short distances of incised tributary valleys. Slopes progressively flatten out downstream to less than 2% (average). The undulating topography renders most farmlands and fields irregular in slope and orientation.

The local climate is cool and humid (Dzikowski et al. 1984). Average annual precipitation at nearby Summerside is 1,039 mm as recorded by the Atmospheric Environment Service of Environment Canada, and average potential evapotranspiration during the growing season, May to September, is 450 mm (Dzikowski et al. 1984). On average, evaporation exceeds precipitation over the growing season, whilst excessive wetness exists during the nongrowing season (Robertson 1964). Winters are generally long, with the major snowmelt events occurring towards the end of March into early April. The average date of the first fall frost is September 30, and of the last spring frost is May 30 (Dzikowski et al. 1984). Intermittent snowfall and snowmelt are characteristic of PEI winters during which the soil is subjected to several freeze/thaw cycles (Edwards 1991).

The soils of the Wilmot River watershed fall predominantly (76%) into the Charlottetown (fine sandy loam) series, expertly classified (MacDougall et al. 1988) as a well drained Orthic Humo-Ferric Podsol derived from fine sandy loam glacial till or residual material. All soils in the Wilmot River watershed are low in natural fertility, low in organic matter (<4%), and practically

![Fig. 1. Map of Prince Edward Island showing location of the Wilmot River watershed as an inset. Reprinted with permission from the GIS mapping unit of the PEI Department of Environment, Energy and Forestry.](image-url)
stone-free in the plough layer. The surface texture is practically uniform: 98% consists of well-drained to moderately well-drained soils (MacDougall et al. 1988).

Sampling and Sample Analysis

The two subcatchments selected for the present study in the eastern upper reaches of the Wilmot River watershed were among the most intensively farmed in the watershed, and were instrumented for year-round recording of discharge via Parshall flumes and automated flow sampling (Burney and Edwards 1994). These subcatchments (Fig. 2) comprised (a) a 140-ha area (designated Curley’s), mainly under potatoes in rotation with barley; and (b) a 416-ha area (designated Mayne’s), mostly under pasture for summer dairy grazing. These subcatchments also had the best year-round accessibility by road. An inventory of land management practices (represented as land use percentage) was done every spring and fall throughout the life of the project (1991 to 2004, Table 1) for each of the subcatchments, using ground surveillance aided by farm boundary maps. Discharge from each subcatchment was recorded at 10-minute intervals. Full-profile stream flow samples were taken from a vertical slit mounted at the exit of each Parshall flume at periods varying from 10 minutes (with rapidly varying flow) to 17 hours (under steady, groundwater-supplied conditions), as described in further detail by Burney and Edwards (1994). The flow samples, once collected, were filtered to remove the sediment and then analyzed for NO$_3$ using an autoanalyzer (Bran-Luebbe Technicon TRAACS 800) to measure NO$_3$ concentration which was either plotted as is or upon conversion to NO$_3$ loading. Grab samples were taken weekly or after major events (as a back-up), whichever was more frequent.

Data Organization and Analysis

With 13 mg of NO$_3$ per litre as a safety reference for freshwaters (for aquatic life) set by the Canadian Council of Ministers of the Environment (CCME 2006), average monthly distributions of NO$_3$ concentration were plotted as shown in Fig. 3a and 3b for the subcatchments for which land management information was also assembled and averaged (Table 1) over the life of the project. The data were used to assess relationships using regression analysis, measuring correlation coefficients between NO$_3$ losses and land management over the same period. The NO$_3$ concentration of each sample was multiplied by the stream flow rate at the time of sampling in order to compute NO$_3$ mass per unit time in the stream discharge. The average of the NO$_3$ mass flow at the start and end of

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**Fig. 2.** Map of the Wilmot River watershed showing the two subcatchments that were the sampling locations - Prince Edward Island. Reprinted with permission from the GIS mapping unit of the PEI Department of Environment, Energy and Forestry.
the period between samples multiplied by the time period was summed over each month on each subcatchment and divided by the subcatchment area. This result provided mass NO$_3$ loss per hectare per month, which was also used to assess monetary loss (dollar equivalent of lost NO$_3$ at prevailing fertilizer market prices), as well as correlation and regression relationships with land management over the study period.

The average monthly water discharge from the subcatchments is shown in Fig. 4a, and the monthly NO$_3$ outflow is shown in Fig. 4b. The yearly total precipitation for the study period, 1991-2004, is shown in Fig. 5 against a 30-year average.

In performing regression analysis to assess the relation of nitrate loading (dependent variable) to land management (independent variable), the data were stratified as percentages of the catchments under a given rotation crop (potatoes, barley, forages, etc.) or crop category (annual crops and long-term cover). The data were also stratified according to season (growing season and nongrowing season) for detailed insight into these relationships.

### Results and Discussion

**Discharge, Nitrate in the Outflow, and Land Use**

As may be noted in Fig. 4a, the distribution of discharge over the year is dominated by two peaks, January and March/April. The first peak represents a winter thaw which commonly lasts for about three days in late January, and the second peak represents the spring snowmelt. These peaks indicate surface flow over a partially thawed surface underlain by a relatively impermeable frost layer. For the period of May through November, discharge in the streams is almost entirely from groundwater.

Nitrate outflow, as seen in Fig. 4b shows similar peaks; although the January peak is higher than the spring snowmelt peak, suggesting that much of the residual nitrate from the previous summer’s fertilizer applications (or organic-source mineralization) is flushed out of the soil during the January thaw. Under certain rainfall conditions, therefore, this phenomenon may well produce apparent anomalies in a year or years subsequent to an N treatment (or amendment) to the soil. The yearly

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### Table 1. Recorded land management (as land use percentage) for the two subcatchments (Curley’s, Mayne’s) studied in the Wilmot River watershed, 1991 to 2004

<table>
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<th>Potatoes (%)</th>
<th>Grain (%)</th>
<th>Hay/pasture (%)</th>
<th>Legumes (%)</th>
<th>Other (%)</th>
<th>Forestry (%)</th>
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<td>0.55</td>
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average NO$_3$ outflow from the Curley’s subcatchment is 22 kg·ha$^{-1}$·year$^{-1}$, and from Mayne’s is 15 kg·ha$^{-1}$·year$^{-1}$, which are of the same orders of magnitude (particularly during October through April) as for work done in New Brunswick by Milburn and Richards (1994) with similar fertilizer inputs for row crops under similar catchment conditions.

Plots of recorded monthly nitrate outflow (nitrate mass flux) (Fig. 4b) from both subcatchments showed parallel peaks for the spring snowmelt period, March to April, then a parallel rise ($r = 0.873; p < 0.01$) in the wet fall/early winter, thus mimicking the recorded monthly discharge (Fig. 4a). This was reflected in significant correlations for Curley’s ($r = 0.735, p < 0.01$) and for Mayne’s ($r = 0.854, p < 0.01$). The significant, recorded differences between subcatchments for both discharge ($t$-test: $p < 0.01$) and nitrate outflow (higher for Curley’s) ($t$-test: $p < 0.01$) can easily be explained on the basis of dominant land use wherein pasture (dominant at Mayne’s) naturally allows less runoff on the basis of better infiltration (Russel 1973) than do row crops (dominant at Curley’s). In the present study, monthly discharge amounts from the two subcatchments were highly correlated ($r = 0.976; p < 0.01$), as may be judged from Fig. 4a, reflecting similar variations under the same precipitation regime. The subcatchments are only about 4 km apart (as may be judged from Fig. 2).

In studying the subcatchments in terms of NO$_3$ concentration, plots of monthly values (Fig. 3) showed considerable flux, reflecting monthly standard deviations (SDs) of 3.42 for Mayne’s and 3.44 for Curley’s, and yearly SDs of 2.74 for Mayne’s and 2.78 for Curley’s. While NO$_3$ concentration values exceeded the 13 mg·L$^{-1}$ freshwater quality guideline (set by the Canadian Council of Ministers of the Environment [2006]) at a 5.7% incidence over 14 years, NO$_3$ concentrations averaged 6.6 mg·L$^{-1}$ overall with a breakdown of 7.0 mg·L$^{-1}$ for Curley’s and 6.2 mg·L$^{-1}$ for Mayne’s. The “incidence of violation” was 6.0% for Curley’s and 5.4% for Mayne’s.
The incidence of violation in the case of Mayne’s, nearly 50% of which is pasture, was most likely attributable to mineralization of cattle urine and faecal matter, since NO₃ applied to pasture is, at best, sparing. Work done by van Bochove et al. (2000) showed that N mineralization can contribute substantially to the NO₃ content of overland flow, even in the cool season.

Regression analyses on the data for nitrate mass (in the streams) and land use (land management) showed no significant relationships—neither overall nor stratified—which might be considered contrary to logical expectation from a situation in which NO₃ application rates vary with crop and crop type. For instance, row crops in this watershed receive an average of 320 kg of NO₃ per hectare. Potatoes receive 440 kg of NO₃ per hectare, and cereals receive 200 kg of NO₃ per hectare (The Prince Edward Island Field Crops Guide 2003). A priori, these contrasts should be reflected in stream nitrate loadings sufficiently to produce measurable variations attributable to regression. Since these variations are not significant, their dampening may have to be explained on the basis of (a) luxury NO₃ uptake (by the crop), which can occur on warm cloudy days, or (b) leaching, which can occur where overland flow is forced to decelerate by undulations in the terrain or by improved flow retardants. Correlation analyses showed estimates of \( r \leq 0.30 \) for row crops and \( r \leq -0.40 \) for forage/pasture (\( p > 0.05 \)). A recent study of nitrates in ground water in the Wilmot River watershed by Savard et al. (2007) attributes annual NO₃ increases (at rates of up to 30%) to the Wilmot River watershed by Savard et al. (2007)

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The average NO₃ concentration (Fig. 3) was least in early spring, which is the period of snowmelt and continual rains that generates increased outflow and consequent NO₃ dilution. A similar NO₃ tendency can be observed in the fall which is customarily wet, sufficiently so to cause a 50% drop in nitrate strength in the outflow. Conversely, both summer and winter showed increases of more than double, reflecting relatively low-volume, concentrated NO₃ outflow in periods when outflow occurs under conditions of freezing or drought. Thus, a strong inverse relationship emerged between water discharge and NO₃ concentration (\( R^2 = 0.82; p < 0.01 \)).

In summary terms, interpretation of Fig. 3 places the discussion at two levels, namely, that of nutrient waste and that of environmental health and safety. In a pure sense, any amount of applied NO₃ that is unused by the crop is excessive; although, this doctrine may be relaxed where unused NO₃ remains in the root zone and is available to the next crop. However, this ideal is beyond practicality because, despite recommended dosages based upon soil tests and planned cropping, farm operators may apply excess NO₃ towards maximum yields. Undoubtedly, every attempt is made to boost spring growth using mobile N sources, but the relative ease of loss and ultimate wastage of applied NO₃ may be blamed on the very characteristic that makes it readily available to the crop, i.e., its solubility.

Nitrate loss means financial loss to the farmer and, in this 14-year study, the assessed loss ranged from $0.15 to $120 per ha per year, averaging $1.70. That these amounts are sufficient to galvanize the average farmer into stricter conservation is doubtful, even at the higher end of the dollar-loss range.

The fear of NO₃ toxicity in water resources invariably finds itself at or near the centre of health care politics. Thus, it also touches potable water from wells situated in or near intensively farmed watersheds. A local survey of 283 rural wells (VanLeeuwen and Keefe 1998) found 7% of these wells with NO₃ levels exceeding drinking water guidelines, but this is outside the scope of the present study. However, from the standpoint of surface water, the results herein reported will allay anxiety for both policymakers and the public at large. The fact that our study of PEI’s most important potato-producing watershed showed only a 5.7% violation incidence should be welcome relief to freshwater resource users in the Wilmot River watershed, and may even provide a basis for watershed planning and usage regulations.

The maxima for both Curley’s and Mayne’s (Fig. 3) were 53% and 60% (respectively) above the freshwater guidelines of 13 mg·L⁻¹, and occurred in the same year, 1996. Since this was a year of moderate rainfall (year 6 of Fig. 5), it may not be explicable purely by coincident low or high precipitation. However, if it be considered that the year prior (year 5 of Fig. 5) received relatively low rainfall and a significant portion of the resident NO₃ was not washed out until the following year, then the relatively high NO₃ concentrations of year 6 might thus be accounted for.

General Observations and Perspective

Towards the simultaneous conservation of NO₃ as a plant nutrient and minimization of aquatic contamination, there is strong local advocacy for substantial replacement of applied inorganic N forms with organic forms including legume-fixed N. And, following heavily NO₃-fertilized crops in any rotation, the use of nitrate “mop-up” crops (MacLeod and Sanderson 2002) is strongly suggested. The use of mop-up crops could even prove valuable in situations where N mineralization is substantial, even as the process of mineralization makes its contribution to cool-season NO₃ overland flow (van Bochove et al. 2000). And, although the practice of mop-up cropping might not win many converts where large-scale commercial exploitation is the intent, it is bound to have public appeal on the basis of a healthy balance between profitability and environmental (specifically freshwater) sanitation.
What this study has succeeded in doing is to show that nitrate outflow from PEI’s most intensively farmed watershed feeds safe NO₃ concentrations (over 94% of the time) into a river that is important to PEI’s inshore fishery on the Wilmot River and the oyster beds in the Bedeque Bay (Fig. 1). The step beyond this study, and to the long-term benefit of the inshore fishery, is further research that looks at the nitrate outflow of the Wilmot River and nitrate level in the affected bay, Bedeque Bay.

Conclusions

1. Recorded NO₃ concentrations lie mostly within the limit of safety for fresh water (“freshwater quality guidelines”) and may allay public disquietude of NO₃ toxicity in the streams under study.

2. The predominance of NO₃ outflow occurs in the dormant season for cropping, during the January thaw and spring snowmelt.

Acknowledgment

The authors gratefully acknowledge the GIS input and mapping by M-L. McCourt of the PEI Department of Environment, Energy and Forestry.

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Received: 23 March 2007; accepted: 26 February 2008.
Influence of Livestock Manure Type on Transport of *Escherichia coli* in Surface Runoff

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*Agriculture and Agri-Food Canada, 5403-1st Ave. South, Lethbridge, Alberta T1J 4B1*

Since livestock manure type may influence transport of *Escherichia coli* (E. coli) in runoff, the choice of which type of livestock manure to apply to cropland may be a potential beneficial management practice (BMP) to reduce and manage *E. coli* in runoff. Four common manure types (beef, dairy, chicken, hog) were applied to a clay loam soil in small runoff boxes, and a rainfall simulator was used to generate artificial runoff. Runoff samples were collected at three successive time intervals (0 to 5, 5 to 15, 15 to 30 min) and analyzed for flow-weighted mean concentrations (FWMC) of *E. coli* as well as mass loss of *E. coli* expressed as a percentage of total *E. coli* applied. Manure treatment had a significant ($p \leq 0.10$) influence on FWMC of *E. coli* in runoff. The FWMC of *E. coli* in runoff for the dairy (33.3 CFU per 100 mL) treatment was similar to the control (3.2 CFU per 100 mL), but *E. coli* concentrations for the beef (955 CFU per 100 mL), chicken (1,134 CFU per 100 mL), and hog (368 CFU per 100 mL) treatments were all significantly greater than the control. The FWMC values were not significantly different among the four manured treatments except for dairy versus chicken manure, where values were significantly lower for dairy manure. Concentrations of *E. coli* were less than the guideline for recreation waters (< 200 CFU per 100 mL) for the control and dairy treatment, but exceeded this guideline for beef, chicken, and hog manures, suggesting that dairy manure may be better than the other three manures for protecting surface water bodies for recreational use. Our study suggests that manure type may be a possible BMP to manage and control FWMC of *E. coli* in surface waters.

**Key words:** *E. coli*, manure type, runoff, rainfall simulation

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**Introduction**

Animal waste management is one type of Beneficial Management Practice (BMP) that may be used to control pollutants such as pathogens in surface runoff (Mostaghimi et al. 2001). Bacteria such as generic *E. coli* are used to indicate pollution of surface waters by pathogenic bacteria that can cause illness in humans that drink these waters or use these waters for recreation. Since livestock manure type may affect runoff of pathogenic bacteria (Crane et al. 1983; Unc and Goss 2004), manure type may have potential to be a BMP to control and manage pathogens in runoff.

The type and number of microorganisms in manure can vary with the animal species, age of animals, the type of bedding used, the method of storage (liquid or solid), and the storage period (Jamieson et al. 2002; Unc and Goss 2004). Manure properties that influence transport of *E. coli* in runoff may include the amount and nature of organic material, amount and nature of mineral material, bedding, moisture content, hydrophobicity, pH, soluble ion content, and type of soluble ions (Reddy et al. 1981; Jamieson et al. 2002; Unc and Goss 2004). When manure is incorporated into soil, interactions of the manure and soil may obscure manure property effects on transport of *E. coli* in runoff. Physical filtration is believed to be the primary process that limits bacterial mobility in soil (Gerba and Britton 1984; Maier et al. 2000). Bacteria range in size from 0.5 to 2 μm and are more subject to filtration than smaller organisms such as viruses (Maier et al. 2000). The water content of manure can influence the hydrology of infiltration and runoff, with liquid manures enhancing surface runoff and solid manures enhancing infiltration (Unc and Goss 2006).

Increasing soluble manure content generally decreases bacterial attachment in soil (Guber et al. 2005a), and has been attributed to increased competition for attachment sites (Guber et al. 2005b). The reduced attachment of bacteria to silt and clay particles in the presence of manure colloids may cause predominantly free-cell transport of manure-borne fecal coliforms in runoff (Guber et al. 2007b). The kinetics of fecal coliform release from manure was found to be similar to the release kinetics for P and organic C (Guber et al. 2006); and *E. coli* release rates changed from first-order to zero-order kinetics after 1 h of rainfall simulation (Guber et al. 2007a).

We are aware of only one study that has examined the influence of livestock manure type on runoff of generic *E. coli*. Soupir et al. (2006) applied liquid dairy manure, solid dairy manure cowpies, and solid turkey manure to a silt loam pastureland without incorporation into the soil. They used a rainfall simulator to generate artificial runoff for large (18.3 x 3 m) runoff plots under initial dry soil conditions and then under subsequent wet soil conditions. They measured flow-weighted concentrations of *E. coli* in runoff at different time intervals. The average *E. coli* concentrations in runoff for the two events were highest under cowpies (5.13 log CFU per 100 mL), followed by liquid dairy (4.26 log CFU per 100 mL), turkey (4.11 log...
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CFU per 100 mL), and then the unamended control (1.15 log CFU per 100 mL). They attributed differences among the treatments to the different initial concentrations in the source material applied to their plots, which was likely due to the unincorporated manure.

We are not aware of studies that have compared the influence of manure type on runoff of E. coli when different manure sources were incorporated into the soil, which is a more realistic condition on annual cropland. In Alberta, manure applied to annual cropland has to be incorporated within 48 hours of application (Province of Alberta 2004). In southern Alberta, the dominant source of manure is from beef cattle. In addition, manure from hogs, poultry, and dairy cattle are also common. If these manure types have an influence on runoff of E. coli, the choice of manure type to be applied to cropland may be a potential BMP to manage bacteria in runoff. We are not aware of any studies that have compared the influence of these four major manure types on runoff of E. coli.

The objective of our study was to compare E. coli in runoff under beef and dairy cattle, hog, and poultry manure. Secondary objectives were to examine the influence of sampling time on E. coli in runoff, as well as the effect of solid versus liquid manure on E. coli in runoff.

**Materials and Methods**

Field experiments were conducted in the summer of 2006 on a clay loam Dark Brown Chernozemic soil (0- to 15-cm depth) obtained from the Lethbridge Research Center. The soil was taken from the field with a shovel, air-dried, and then sieved through a 2-mm sieve. Runoff soil boxes (100-cm length x 20-cm width x 5-cm depth) were constructed of stainless steel to hold the soil during rainfall simulations. The runoff soil boxes consisted of five individual trays to hold soil and manure for each treatment. Soil was packed into each soil tray to a bulk density of 1.10 g·cm⁻³ so that the top of the soil was level with the runoff tray.

The experimental treatments were manure applied from beef cattle, dairy cattle, chicken, and hog, as well as an unamended control. There were five replications for each of the five treatments. The solid beef cattle manure was obtained from a storage pile adjacent to the Lethbridge Research Center feedlot and consisted of feedlot pen floor manure with wood chips. The wood chip bedding was a mixture of sawdust and bark peelings derived from 80% lodgepole pine (Pinus contorta var. latifolia Engelm.) and 20% white spruce [Picea glauca (Moench) Voss]. The liquid dairy manure was obtained from a concrete storage lagoon at the dairy barns of the Lethbridge Research Center. The solid poultry manure was obtained from a local commercial poultry operation. We don’t know how long the poultry manure had been stored, but its low water content (Table 1) indicated it was likely stored for a considerable length of time. The

| TABLE 1. Selected physical and chemical properties of the four manure types and soil |
|----------------------------------|--------------------|----------------|----------------|
| Manure/soil                      | Application rate of manure (kg or mL) | Total E. coli content (log CFU/mL) | Water content (kg per soil) |
| Beef                             | Solid              | 2.21±0.32,1.5 (1.1) | 9.08±0.05 | 6.4±0.1,5.7 (5.7) |
| Chicken                          | Liquid             | 3.31±0.16,8.3 (8.0) | 6.7±0.04 | 5.0±0.5,4.7 (4.5) |
| Dairy                            | Solid              | 3.24±0.09,1.5 (1.5) | 6.6±0.01,5.7 (5.7) |
| Hog                              | Liquid             | 3.31±0.16,8.3 (8.0) | 6.7±0.04 | 5.0±0.5,4.7 (4.5) |
| Soil                             |                    | -               | -            | -                |

a. kg wet for solid, mL for liquid.
\( EC = \) electrical conductivity ratio.
\( SAR = \) sodium adsorption ratio.
Values in parentheses are dry weight (kg) of manure applied, assuming 1 mL = 2.1 L.
Livestock Manure Type Influences E. coli in Runoff

Liquid swine manure was obtained from a storage lagoon at a local hog operation. The liquid manures in the dairy and hog lagoons were not mixed prior to collection. Manure samples were obtained in July, 2006. Solid manure samples were stored in plastic bags, and liquid manure samples were stored in 20-L plastic containers at 4°C between 4 to 17 days prior to application to the runoff boxes. Subsamples of manure were taken from the stored manure source and analyzed prior to application to each of the five replicates.

Manure was applied to soil at typical rates applied by producers in the area. The typical application rate (wet basis) for beef cattle manure to cropland in the Lethbridge area is 75 Mg·ha⁻¹ (Porcupine Coral Cleaners personal communication 2006). A similar rate was used for solid chicken manure. Typical rates for liquid dairy and swine manure in the Lethbridge area are 4,000 imperial gallons per acre or 45,000 L·ha⁻¹ (McKenzie personal communication 2006). The appropriate weight or volume of moist manure for each manure type was applied to one soil tray by hand and incorporated to a depth of 5 cm using a hand trowel. A portable Guelph rainfall simulator (Tossell et al. 1987) was used to apply deionized water to the soil trays at a rainfall intensity of 70 mm·h⁻¹. After runoff commenced, total cumulative runoff at 0 to 5, 5 to 15, and 15 to 30 min intervals was collected, and subsamples were taken for bacterial analyses. Overall, there were 5 treatments by 5 replications by 3 time intervals for a total of 75 runoff samples collected. The volume of runoff water for each of the three time intervals was measured.

Runoff water samples were analyzed for E. coli using the Colilert method (IDEXX Laboratories, Westbrook, ME, U.S.A.), and results were expressed as MPN (Most Probable Number) or CFU (Colonies Forming Units) of E. coli per 100 mL of water. Manure samples were extracted with deionized water to determine the E. coli content of the water. Various manure:water ratios (1:10, 1:100, 1:1000) were used to obtain countable concentrations of E. coli. Concentrations of E. coli in manure were converted to CFU per gram of dry manure using the water content of the manure and the manure:water ratio.

The Colilert test relies on the substrates O-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG) to detect total coliforms and E. coli, respectively. The presence of coliforms is indicated by a change in the medium from clear to yellow, while the presence of E. coli is determined by fluorescence under long-wave (366 nm) ultraviolet light. The Colilert method has been compared with the standard membrane filtration method for various media (freshwater, soil, food, and feces) and has been found to accurately detect total coliforms and E. coli (Edberg et al. 1988; Rice et al. 1990, 1991; Clark et al. 1991; Frampton and Restaino 1993; Muirhead et al. 2004). The Colilert method for fresh water was approved by the United States Environmental Protection Agency for total coliforms in 1989, and for detection of E. coli in June 1992 (Palmer et al. 1993), and is a proposed method for total coliforms and E. coli (APHA 1998).

Flow-weighted mean concentrations (FWMC) of E. coli in runoff water were calculated by dividing the total mass of bacteria in runoff by the total volume of runoff. The mass loss of E. coli in runoff as a percentage of the total amount applied was calculated by dividing the total mass of E. coli in runoff by the total mass of E. coli applied (for each replicate), and then multiplying by 100.

The water content of the manure was determined by oven-drying a field-moist subsample of manure at 60°C, and then determining the oven-dry weight. The pH, electrical conductivity (EC), and sodium adsorption ratio (SAR) of the manure were determined on 1:5 manure and water extracts. Soluble Ca and Mg were analyzed using atomic absorption spectroscopy, and Na was determined using flame emission spectroscopy (Model AAS; PerkinElmer, Wellesley, Mass.) (Wright and Stuczynski 1996). Nitrate and ammonium in the manure were extracted using a 1:20 ratio of 10 g of manure and 200 mL of 2 M KCl after shaking at low speed for one hour. Ammonium N was determined using the Berthelot reaction on the autoanalyzer (Technicon Industrial Systems 1973). Nitrate N was determined on the autoanalyzer using the copper-cadmium method (Technicon Industrial Systems 1978). Manure samples were finely ground to pass a 150-μm sieve, and total N and C were determined using the Dumas automated combustion technique (McGill and Fiqueiredo 1993), using a CNS analyzer (Carla Erba, Milan, Italy).

Statistical Analyses

The influence of manure type, time of sampling, and the possible two-way interaction on FWMC and mass loss of E. coli in runoff were analyzed using SAS (SAS Institute 1989). A mixed model analysis with the REPEATED statement for time of sampling was used for the analyses (Littell et al. 1998). Different covariance model types were tested to obtain the best covariance structure for the mixed model. A mixed model analysis was conducted on the log-transformed FWMC data to make the residuals normal and the variances uniform. For the percentage loss data, the data were ranked by replicate using the RANK procedure in SAS, and then the mixed model analysis was conducted on the ranked data (Conover and Imann 1981). A mixed model analysis was conducted on the untransformed runoff volume data since no log transformation was required. Comparisons among means were conducted with a Tukey-Kramer test, and were considered significant at the p ≤ 0.10 level. An ESTIMATE statement in SAS was used to determine the influence of liquid versus solid manure on FWMC, and mass loss of E. coli in runoff. Correlation analysis (p ≤ 0.05) was conducted to ascertain possible relationships between manure properties, runoff volume, and FWMC and mass loss of E. coli in runoff.
Results and Discussion

Manure Properties

Beef manure had the highest values for pH and SAR; chicken manure had the highest values for EC, total C, and total N; dairy manure had the highest values for NO₃-N; and hog manure had the highest values for water content, *E. coli* concentration, and NH₄-N (Table 1). Concentrations of *E. coli* in manure were highest for hog, followed by chicken, dairy, and then beef. Concentrations of pathogenic bacteria are likely to be greater in hog and poultry manure than in cattle manure (Unc and Goss 2004).

Mean concentrations of *E. coli* in beef manure (2.21 log CFU-g⁻¹ of dry manure) at the time of application after storage at 4°C for 4 to 17 days (Table 1) were considerably less than concentrations (7.61 log CFU-g⁻¹ of dry manure) reported in fresh pen manure taken from this same feedlot in the summer and plated within 4 hours (Miller et al. 2003). Storage of manure enhances die-off of *E. coli*, and follows simple first-order kinetics (Meals and Braun 2006). Although our manure samples were stored at 4°C to minimize die-off of *E. coli* during storage, considerable die-off can still occur at this temperature. Kuvda et al. (1998) reported that the concentration of *E. coli* 0157:H7 in cattle feces incubated at 4°C decreased by 2 logs 48 h after inoculation but remained constant thereafter. However, feedlot pen manure is often cleaned from pens and stored for periods even longer than 17 days, so storage of our manure prior to application is a common practice in the industry.

Mean concentrations (3.42 log CFU-g⁻¹ of dry manure) of *E. coli* in chicken manure in our study (Table 1) were comparable to concentrations (3.48 log CFU-g⁻¹) reported for turkey manure that was stored for three weeks (Soupir et al. 2006). Mean concentrations (3.31 log CFU-g⁻¹ of dry manure) of *E. coli* in dairy manure in our study (Table 1) were considerably lower than values (5.00 to 6.50 log CFU-g⁻¹ of dry manure) reported for fresh dairy manure by others (Guber et al. 2007a; Meals and Braun 2006; Soupir et al. 2006). Mean concentration (4.89 log CFU-g⁻¹ of dry manure) of *E. coli* in hog manure in our study was within the range of values (0 to 6.11 log CFU-g⁻¹ of dry manure) reported by Côté and Quessy (2005).

Influence of Manure Type and Sampling Time on Runoff Volume

Manure type had a significant effect on runoff volume, where values were 19 to 29% lower for chicken manure than the other four treatments (Table 2). Runoff volumes were significantly greater for each of the three successive sampling intervals (Table 2). The volume of runoff was significantly greater for liquid (2,234 mL) than solid (1,912 mL) manure, and was consistent with the higher water content of liquid than solid manures (Table 1). Liquid manure also has a higher potential than solid manure to clog finer pores in soils with no structure or macropores (Unc and Goss 2006), and may have contributed to enhanced runoff under liquid manure. We used repacked soils in our study, which had no structure and no macropores. Most field medium- to fine-textured soils have well-developed structure and contain macropores. In well-structured soils containing macropores, liquid manure favours macropore flow over matrix flow, whereas solid manure favours matrix flow over macropore flow (Unc and Goss 2006).

Influence of Manure Type and Sampling Time on Runoff of *E. coli*

The FWMC and mass loss of *E. coli* in runoff for the three sampling intervals is shown on Fig. 1. There was no significant interaction of manure type x time on *E. coli* concentration or mass loss in runoff (Table 2). In comparison, Soupir et al. (2006) reported that temporal variation of *E. coli* concentrations in rainfall simulation runoff was dependent on manure type, suggesting a possible interaction of manure type with time. However, they conducted their study on much larger (18.3 x 3 m) runoff plots than in our study, and used undisturbed field soils (silt loam) that likely had well-developed structure and macropores.
Livestock Manure Type Influences E. coli in Runoff

Manure type had a significant ($p \leq 0.10$) effect on the FWMC of E. coli in runoff (Table 2). Mean FWMC of E. coli was significantly greater for three manure-amended treatments (beef, chicken, hog) than the unamended control, indicating that application of these manures to cropland increases the potential for increased concentrations of E. coli above background levels. In contrast, mean FWMC of E. coli in runoff was not significantly different between the dairy manure treatment and the control, indicating a low potential for increased concentrations of E. coli from dairy manure above background levels compared with the other three manure types. Soupir et al. (2006) reported that average flow-weighted concentrations of E. coli in runoff from solid dairy cowpies was significantly greater than the control, but that concentrations of E. coli from unincorporated liquid dairy and turkey amended fields were similar to the control.

For comparisons among the four amended treatments (Table 2), mean FWMC of E. coli were similar among all two-way comparisons except between dairy and chicken, where concentrations were significantly greater for the chicken than dairy treatment. Soupir et al. (2006) reported contrasting results where they found no significant difference in average flow-weighted concentrations of E. coli in runoff between turkey and liquid dairy manure treatments. They also reported that E. coli concentrations were significantly greater under solid dairy cowpies than turkey litter, and there was no difference between solid dairy cowpies and liquid dairy manure.

Mean FWMC values of E. coli in runoff for the control and dairy treatment (Table 2) were less than the water quality guideline (<200 CFU per 100 mL) for recreational waters (Health Canada 1992). In contrast, mean FWMC values exceeded this guideline by 5.7 times for chicken manure, 4.8 times for beef manure, and 1.8 times for hog manure. Therefore, our results suggest that dairy manure application may be the best BMP for maintaining surface water quality for recreational use with respect to E. coli. In comparison, concentrations of E. coli in runoff from liquid dairy, solid dairy cowpies, and solid turkey litter in the study by Soupir et al. (2006) were >200 CFU per 100 mL, whereas runoff from the control plots was below this value.

Manure treatment had no significant effect on mass loss of E. coli in runoff (Table 2). The percentage of applied E. coli lost in runoff ranged from 0.1 to 5.3%.

### Table 2. Influence of manure treatment, sampling time, and manure type (liquid vs solid) on flow-weighted mean concentration (FWMC) of E. coli, loss of E. coli in runoff as a percentage of applied, and on volume of runoff.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FWMC of E. coli in runoff (CFU per 100 mL)</th>
<th>Mass loss of E. coli in runoff (%)</th>
<th>Volume of runoff (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check</td>
<td>3.2±1.3 bc</td>
<td>—</td>
<td>2123±320 a</td>
</tr>
<tr>
<td>Dairy</td>
<td>33.3±9.1 bc</td>
<td>0.5±0.2 a</td>
<td>2248±334 a</td>
</tr>
<tr>
<td>Beef</td>
<td>955±298 ab</td>
<td>5.3±4.5 a</td>
<td>2077±272 a</td>
</tr>
<tr>
<td>Chicken</td>
<td>1134±578 a</td>
<td>0.7±0.4 a</td>
<td>1747±282 b</td>
</tr>
<tr>
<td>Hog</td>
<td>368±86.8 ab</td>
<td>0.1±0.03 a</td>
<td>2221±327 a</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–5 min</td>
<td>482±159 a</td>
<td>0.2±0.1 b</td>
<td>783±44.9 c</td>
</tr>
<tr>
<td>5–15 min</td>
<td>791±389 a</td>
<td>0.8±0.3 a</td>
<td>2054±56.4 b</td>
</tr>
<tr>
<td>15–30 min</td>
<td>300±163 b</td>
<td>3.8±3.2 ab</td>
<td>3373±99.6 a</td>
</tr>
<tr>
<td>Liquid</td>
<td>200±55.1 a</td>
<td>0.3±0.1 a</td>
<td>2234±229 a</td>
</tr>
<tr>
<td>Solid</td>
<td>1045±320 a</td>
<td>2.8±2.1 a</td>
<td>1912±195 b</td>
</tr>
<tr>
<td>Treatment</td>
<td>&lt;0.0001 $\dagger$</td>
<td>0.2278</td>
<td>0.0080</td>
</tr>
<tr>
<td>Time</td>
<td>0.0082</td>
<td>0.0059</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatment x Time</td>
<td>0.2688</td>
<td>0.4221</td>
<td>0.2443</td>
</tr>
<tr>
<td>Liquid vs Solid</td>
<td>0.2679</td>
<td>0.3399</td>
<td>0.0030</td>
</tr>
</tbody>
</table>

$^\dagger$ Mean ± standard errors followed by different lower case letters (by column) are significantly different at $p \leq 0.10$.

$^\dagger$ Probability ($p$) of F statistic occurring by chance. Values $\leq 0.10$ indicate a significant effect.
Other researchers have reported annual mass losses for fecal coliforms in runoff from manured land ranging from 2 to 23% (Robbins et al. 1971), 0.1% (McCaskey et al. 1971), 0.1 to 6.7% (Kunkle 1979), and 0.1 to 90% (Crane et al. 1983). Percentage losses of fecal coliforms in runoff were generally highest immediately after application, and decreased dramatically with increased residence time of manure in the soil. Overall, our ranges in mass losses were within the range of values reported by others.

Although there was no significant difference between solid and liquid manure for mean FWMC and mass loss of *E. coli* in runoff (Table 2), the nonsignificant trend was for higher *E. coli* in runoff under solid than liquid manure. McCaskey et al. (1971) also reported that mass loss of fecal coliforms was greater for solid than liquid dairy manures. In comparison, Soupir et al. (2006) reported no significant difference in concentrations of *E. coli* in runoff between liquid dairy manure, solid dairy manure, and solid turkey manure. Since liquid manure favours runoff over infiltration and solid manure favours infiltration over runoff (Unc and Goss 2004, 2006), higher *E. coli* in runoff would be expected under liquid than solid manure if simply based on hydrological partitioning. However, other physical-chemical factors such as bacterial attachment, filtration, mechanical filtration (Guber et al. 2005a), and other factors may also be important in influencing *E. coli* in runoff under liquid and solid manure application.

Sampling time had a significant effect on FWMC and mass loss of *E. coli* in runoff (Table 2), and was consistent with previous findings reporting that time dependent processes are important in the transfer of bacteria from soil to runoff (Crane et al. 1983). Peak values occurred at the second (5 to 15 min) sampling interval for FWMC, and at the third (15 to 30 min) sampling interval for percentage loss of *E. coli*, and may be related to gradual dissolution of manure lumps and delayed release of *E. coli*. Soupir et al. (2006) examined the temporal distribution of *E. coli* in runoff over 3 h for dry soil conditions, and over 1 h for wet soil conditions. They found that peak *E. coli* concentrations were the highest for the first sampling interval, or else concentrations increased with time as runoff intensity increased and peak concentrations occurred at later sampling intervals. Correlation analyses of the volume of runoff versus concentration of *E. coli* in runoff for each manure type and replicate and time interval indicated no significant relationships between runoff volume and *E. coli* in our study. Guber et al. (2007a) reported that dilution and loss to infiltration were the dominant mechanisms causing a decrease in *E. coli* during runoff.

To determine any potential influence of manure properties on runoff of *E. coli*, correlations were conducted between FWMC and mass loss of *E. coli* in runoff versus selected manure properties such as *E. coli* concentration in manure, mass of *E. coli* applied in manure, water content of manure, pH, EC, SAR, total N, total C, NH₄-N, and NO₃-N. No significant (*p > 0.05*) relationships were found among any of the correlations (data not shown), suggesting none of these manure properties influenced *E. coli* in runoff. However, the absence of any relationships may have also been due to the small (*n = 4*) sample size used for correlation analyses.

The greater the concentration of bacterial pathogens in manure, the more likely some will be transported (Goss et al. 2002). The *E. coli* concentration in manure followed the sequence: hogs>chicken>beef>dairy. The *E. coli* concentration in runoff followed the sequence: chicken>beef>hog>dairy. If *E. coli* in runoff was simply related to the concentration of *E. coli* in the original source material, then *E. coli* in runoff should have been highest for hog manure, and followed the above sequence that we found in manure. Since this did not occur and there was no significant correlation between *E. coli* in manure and *E. coli* in runoff, we concluded that *E. coli* in runoff was not related to concentrations in the manure, and that interactions of the manure with soil upon incorporation may have obscured this effect. In contrast, Soupir et al. (2006) reported that *E. coli* concentrations in runoff were primarily due to the different initial bacterial concentrations in the source manure applied to their plots. However, they did not incorporate the manures into the soil in their study (and we did), which may account for them finding a link between *E. coli* in runoff and source manure.

We are unsure as to why manure type had a significant influence on FWMC of *E. coli* in runoff but had no affect on mass loss of *E. coli* (Table 2), and further research is required to investigate the mechanisms involved. Manure is a heterogeneous complex mixture containing water, soluble inorganic and organic chemicals, soil, microorganisms, and dietary fibre. Previous research has shown that increased dissolved manure content resulted in decreased attachment of *E. coli* to soil (Guber et al. 2005a, 2005b), particularly to those soils with high silt, clay, and coated sand fractions, and this may enhance free-cell transport of manure-borne *E. coli* in runoff (Guber et al. 2007b). We found a nonsignificant (*p = 0.06*) but strong positive correlation (*r = 0.94*) between the dry weight of manure applied (Table 1) and FWMC of *E. coli* (Table 2), suggesting that increasing solid manure content may contribute to greater *E. coli* concentrations in runoff. The decrease in bacterial attachment in the presence of manure may be caused by modification of soil mineral surfaces by soluble organic and inorganic constituents in manure, adsorption of bacteria on manure particulates, competition of dissolved organic matter and bacteria for adsorption sites, or bacterial surfaces being modified by dissolved organic matter (Guber et al. 2005a).

**Conclusions**

Both concentration and mass loss data are required to evaluate nonpoint source pollution (Magette 2001).


Province of Alberta. 2004. Agricultural operation practices act and regulations. Alberta Queen's Printer, Edmonton, AB.


Received: 27 September 2007; accepted: 2 May 2008.
Transport of Lithium Tracer and \textit{E. coli} in Agricultural Wastewater Treatment Wetlands

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Agricultural waste must be managed effectively to protect surface and groundwater resources, as well as human health. Constructed wetlands can provide a low-cost environmentally acceptable method for the treatment of agricultural wastewater. An ionic tracer (Lithium chloride [LiCl]) and a biotracer (a naladixic acid-resistant strain of \textit{Escherichia coli}) were injected into six pilot-scale constructed wetlands treating dairy wastewater: three surface-flow (SF) wetlands and three subsurface-flow (SSF) wetlands. Each wetland was 3.9-m long and 1.7-m wide. Residence time distribution functions were calculated for each wetland to investigate the hydraulic behaviour of each system during winter and summer conditions. During the summer study, the mean residence times for SF wetlands 2, 4, and 6 were 12, 16, and 14 days, respectively, while the mean residence time for SSF wetlands 1, 3, and 5 were 23, 18, and 22 days, respectively. The longitudinal dispersion coefficients were in the order of $10^{-6} \text{ m}^2 \text{s}^{-1}$ for each wetland during the summer and winter. The mean residence time for SF wetlands 2, 4, and 6 during the winter study were 8, 10, and 10 days, respectively, while the mean residence time for SSF wetlands 1, 3, and 5 were 8, 9, and 10 days, respectively. \textit{E. coli} effluent peaks often occurred prior to Li peaks, suggesting that bacteria may be motile within the wetland environment. This study suggests that dispersion is an important mass transport process in both SF and SSF wetlands. Long-term operation of SF and SSF treatment wetlands may cause reduced retention times and treatment efficiency due to organic matter accumulation and channelling. Cold winter temperatures may also increase the survival of bacteria within treatment wetland systems, decreasing the wetland's ability to reduce bacteria concentrations during the winter months.

Key words: constructed wetlands, hydraulics, \textit{E. coli} NAR, lithium tracer, biotracer

Introduction

Natural wetlands are located in a number of topographical areas, all of which are flooded during the majority of the year. Wet conditions create a favourable environment for an abundance of biological activity. Wetlands are capable of degrading many waterborne contaminants such as those found in agricultural wastewater (Kadlec and Knight 1996). Agricultural wastewaters contain a number of pollutants such as bacteria, pathogenic microorganisms, biochemical oxygen demand (BOD), pesticides, and nutrients, all of which have great potential to degrade water quality (Kadlec and Knight 1996).

Constructed wetlands, which mimic natural wetlands, have proven to be effective for wastewater treatment (NRCS 2002). Studies have demonstrated that although treatment efficiencies may vary, constructed wetlands are capable of treating agricultural wastewater year-round, even in cold climates (Smith et al. 2005). They are also considered to be a viable treatment option, especially where other conventional methods are not suitable (Karathanasis et al. 2003).

Tracer studies can generate valuable information that helps describe the hydraulics of constructed or natural wetlands. This aids in the prediction of wetland treatment (Hodgson et al. 2004). Flow paths and velocities, retention times, and dispersion in ground and surface water systems are often determined by injecting a conservative chemical tracer or dye (Dierberg and DeBusk 2005). Lithium chloride (LiCl) has been used as a conservative tracer in previous studies to determine the hydraulic retention time of large lakes (Nickus and Thies 2001), and to assess the hydraulics of treatment wetlands (Rash and Liehr 1999; Dierberg and DeBusk 2005). Dierberg and DeBusk (2005) concluded that LiCl behaves more conservatively than rhodamine within vegetated wetlands. In their study, photolysis and sorption losses of lithium throughout the wetland were less than rhodamine losses. The amount of tracer recovered varied, but the overall recovery did not affect the accuracy of key hydraulic parameters (Dierberg and DeBusk 2005).

Biological tracers have been used to investigate the behaviour of bacteria and viruses within ground and surface water systems. Of specific interest when investigating waste-receiving waters is the naladixic acid-resistant strain of \textit{Escherichia coli} (\textit{E. coli} NAR). \textit{E. coli} NAR behaves similarly to other strains of \textit{E. coli}, but it
occurs rarely in the environment (Shadford et al. 1997). 

*E. coli* NAR has been isolated and used as a bior tracer in several studies within river systems (Jamieson et al. 2005), septic systems (Shadford et al. 1997), and agricultural watersheds (Joy et al. 1998). To date, however, it has not been applied to wetlands that are used for wastewater treatment. Performing a conservative chemical and *E. coli* NAR tracer study simultaneously can provide useful information on the wetland’s hydraulic and *E. coli* transport processes.

This paper presents *E. coli* NAR and Li tracer data obtained from summer and winter tracer studies performed within six pilot-scale treatment wetlands in Truro, Nova Scotia in 2005. The objective of this study was to assess *E. coli* transport within surface-flow (SF) and subsurface-flow (SSF) wetlands receiving dairy wastewater, and to determine the wetlands’ hydraulic characteristics (mean residence time and longitudinal dispersion coefficient). Seasonal variability and the differences between the SF and SSF wetlands were also examined.

**Materials and Methods**

**Experimental Facility**

The six pilot-scale constructed wetlands that were utilized for this study are located at the Bio-Environmental Engineering Centre (BEEC) in Truro, Nova Scotia. They included three SF wetlands and three SSF wetlands that are contained within concrete foundations. Each wetland was 3.9-m long and 1.7-m wide, and each was protected by a greenhouse cover for gas emission monitoring (Fig. 1). The SF wetlands contain two deep zones (0.8-m deep) separated by a shallow zone (0.15-m deep), and the SSF wetlands were filled with 0.65 m of washed gravel (2-cm diameter; 39% porosity) and 0.1 m of pea stone (41% porosity). Cattails were planted in June 2005 within the SSF wetlands and within the shallow zones of the SF wetlands. Each wetland was loaded with approximately 100 L of dairy wastewater daily, resulting in a theoretical hydraulic retention time of about 20 days. Inlet flow was regulated using a peristaltic pump calibrated by pump time, and outflow was measured by tipping buckets wired to a Campbell Scientific data logger. Daily evapotranspiration rates were determined by monitoring the relative humidity above the inlet and outlet of each wetland while air was forced through the greenhouse cover at a rate of 30 m·s⁻¹. Wastewater flow rates are summarized in Table 1, and Table 2 lists the average contaminant concentrations for the influent dairy wastewater.

**Tracer Study Procedures**

The following procedures were performed for both summer and winter tracer studies. An *E. coli* NAR broth culture was prepared by inoculating 200 mL of tryptic soy

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**Table 1. Wetland water budget: outflow, ET, calculated inflow, and HRT from SF and SSF wetlands**

<table>
<thead>
<tr>
<th></th>
<th>Outflow (L·d⁻¹)</th>
<th>ET (L·d⁻¹)</th>
<th>Inflow (L·d⁻¹)</th>
<th>HRT (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Avg a</td>
<td>SD</td>
<td>Avg b</td>
<td>SD</td>
</tr>
<tr>
<td><strong>Summer 2005</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>73</td>
<td>23</td>
<td>18 (3)</td>
<td>14</td>
</tr>
<tr>
<td>SSF</td>
<td>75</td>
<td>23</td>
<td>15 (2)</td>
<td>12</td>
</tr>
<tr>
<td><strong>Winter 2005</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>74</td>
<td>36</td>
<td>0 (0)</td>
<td>6</td>
</tr>
<tr>
<td>SSF</td>
<td>81</td>
<td>29</td>
<td>0 (0)</td>
<td>5</td>
</tr>
</tbody>
</table>

- ET = evapotranspiration; HRT = hydraulic retention time; SF = surface flow; SSF = subsurface flow; Avg = average; SD = standard deviation.
- Number in brackets represents average daily ET loss in millimetres (mm).
- Inflow = average outflow + average ET.
- HRT = (wetland volume) / inflow.

**Table 2. Dairy farm wastewater characteristics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Average influent concentration (mg·L⁻¹)</th>
<th>Average loading rate (kg·ha⁻¹·d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD</td>
<td>160</td>
<td>24</td>
</tr>
<tr>
<td>TSS</td>
<td>620</td>
<td>94</td>
</tr>
<tr>
<td>TKN</td>
<td>266</td>
<td>40</td>
</tr>
<tr>
<td>TP</td>
<td>49</td>
<td>7</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>883,000 b</td>
<td>1.3 E+12 c</td>
</tr>
</tbody>
</table>

- BOD = biological oxygen demand; TSS = total suspended solids; TKN = total Kjeldahl nitrogen; TP = total phosphorous.
- Units for *E. coli* are CFU/100 mL.
- Units for *E. coli* are CFU·ha⁻¹·d⁻¹.
broth with *E. coli* NAR. The soy broth contained a 200 mg·L⁻¹ concentration of naladixic acid for the selective growth of *E. coli* NAR. The culture was incubated at 37°C and agitated at 200 rpm for approximately 16 h; a 20-mL sample of the broth culture was measured, using a sterile pipette, into seven 500-mL sample bottles. The culture was then diluted with tap water to fill a 500-mL bottle. One bottle was analyzed to determine the initial *E. coli* NAR concentration, and the other six were used as tracers. The initial concentration of *E. coli* NAR during the summer study was 8x10⁸ colony forming units (CFU) per 100 mL, and 6x10⁸ CFU per 100 mL for the winter study.

Seven LiCl solutions were prepared in sample bottles by mixing 10 g of LiCl with 100 mL of deionized water. One solution was saved to measure the initial Li concentration, and the other six were used as tracers. The winter tracer study commenced on 12 December 2005 and the summer tracers were introduced on 18 August 2005. The LiCl and *E. coli* NAR solutions were injected into the inlet pipe of the six wetlands. A high pressure hose was used to flush the tracers down the inlet pipes, directly into the wetlands. Approximately 1.5 L of flushing water was used for each wetland. Effluent samples were collected daily for the first week, and then three times per week for an additional two weeks. Samples were taken during the summer study from 18 August 2005 through 20 October 2005, and samples were taken during the winter tracer study from 12 December 2005 through 2 January 2006. Fewer samples were taken during the winter study because *E. coli* NAR effluent concentrations dropped off more quickly than in the summer. Results from the summer provided information with respect to appropriate sampling frequencies.

Samples were analyzed for concentrations of Li and *E. coli* NAR. The Li concentrations were measured using an Atomic Absorption (AA) Spectrometer with a detection limit of 0.01 mg·L⁻¹. Effluent samples were analyzed for *E. coli* NAR using the membrane filtration technique (APHA 2000). The *E. coli* NAR were enumerated using HACH m-ColiBlue24 broth during the summer tracer study. Difco mTEC agar was used during the winter tracer study. Both the m-ColiBlue24 broth and the mTEC agar were spiked with 200 mg/L of naladixic acid. Plates were incubated at either 35°C (m-ColiBlue24) or 44°C (mTEC) for 24 h, and then plate counts were performed.

M-ColiBlue24 broth spiked with naladixic acid achieves the selective growth of *E. coli* NAR; however, total coliforms may also appear. mTEC promotes the selective growth of *E. coli* only. When the summer tracer study was complete, a laboratory experiment was performed to test each selective growth medium (m-ColiBlue24 and mTEC). Both plate counts produced similar results (within the same order of magnitude), but colonies on the mTEC plates were more visible and therefore easier to count. The mTEC agar eliminates the possibility for total coliform growth; therefore, mTEC agar was used during the winter tracer study.

### Calculations

The Li data obtained from both studies were used to determine the mean hydraulic residence time for each wetland. The function $E(t)$ represents the residence time distribution (RTD) function which describes the amount of time that a particular fluid element spends in the system (Fogler 1992):

$$E(t) = \frac{C(t)}{\int C(t)dt} \quad (1)$$

$C(t)$ represents the effluent tracer concentration at time $t$, and the integral in the denominator is the area under the $C(t)$ curve. The mean residence time, $t_m$, can then be calculated by taking the first moment of the RTD function, as follows:

$$t_m = \int tE(t)dt \quad (2)$$

Variance ($\sigma^2$), or the square of the standard deviation, was then calculated to obtain a measure of the degree of “spread” of the data distribution. Variance ($\sigma^2$) was determined by taking the second moment about the mean residence time (Fogler 1992):

$$\sigma^2 = \int (t - t_m)^2 E(t)dt \quad (3)$$

Variance was calculated for each wetland, and was then used to calculate a dispersion coefficient ($D$). Variance as calculated above is in units of time; this must be converted to variance in space before calculating longitudinal dispersion coefficients (Apello and Postma 1994):

$$\sigma^2 = \frac{\sigma^2 x^2}{t_m} \quad (4)$$

where $x$ is the total length of the wetland (3.9 m).

Variance in space was then used to calculate a longitudinal dispersion coefficient $D_D$ (D) for each SF and SSF wetland (Apello and Postma 1994):

$$D_D = \frac{\sigma^2 x^2}{2t_m} \quad (5)$$

The amount of tracer that was recovered from each wetland was calculated for the summer and winter studies. Tracer concentrations were assumed to be constant throughout the day. The tracer concentration data and daily flow volumes ($Q$) were used to calculate the mass of tracer that was recovered ($MR$) during each study:
\[ M(t) = C(t)Q(t) \]  
\[ MR = \int M(t)dt \]

Simpson’s rule was used for the integration technique (Fogler 1992).

Results & Discussion

Figure 2 displays the *E. coli* NAR observed data from the summer (a) and winter (b) studies. *E. coli* NAR concentrations in the effluent are presented in CFU 100 mL\(^{-1}\).

Time series plots of Li concentrations within the wetland effluent during the summer (a) and winter (b) studies are provided in Fig. 3.

Table 3 displays the mean residence time and dispersion coefficients that were calculated for each SF and SSF wetland during the summer and winter tracer studies.

The percent mass recoveries associated with Li and *E. coli* NAR from each wetland during the summer and winter studies are presented in Table 4.

**E. coli NAR**

During the summer study, *E. coli* NAR first appeared in effluent samples after 3 and 4 days in the SF wetlands,

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**Fig. 2.** *E. coli* NAR effluent tracer concentrations with time for SF wetlands 2, 4, and 6, and SSF wetlands 1, 3, and 5; (a) summer study (b) winter study.
peak within the SF wetlands; the wastewater flow would encounter fewer obstacles within the SF wetland than the SSF wetland. Flow through the SSF wetlands must follow a tortuous path through the porous media, which can explain the lag in \textit{E. coli} NAR peak concentrations. The \textit{E. coli} NAR would also experience increased die-off because of the extended retention time within the SSF wetlands, hence the lower peak concentrations. Because the cattails were not well established, the SSF wetlands would provide more efficient removal mechanisms than the SF wetland. Pores within the pea stone and gravel in the SSF wetland create an ideal environment for trapping suspended solids, and \textit{E. coli} tend to associate with solid particles (Jamieson et al. 2005). In summary, the lower \textit{E. coli} NAR effluent concentrations observed may be attributed to a longer retention time, increased die-off, and removal mechanisms within the SSF wetlands.

![Graph](image)

**Fig. 3.** Lithium effluent tracer concentrations with time for SF wetlands 2, 4, and 6, and SSF wetlands 1, 3, and 5; (a) summer study (b) winter study.

<table>
<thead>
<tr>
<th>Wetland</th>
<th>Mean residence time $t_m$ (days)</th>
<th>Dispersion coefficient $D$ (m$^2$·s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Summer</td>
<td>Winter</td>
</tr>
<tr>
<td>SF2</td>
<td>12.0</td>
<td>8.5</td>
</tr>
<tr>
<td>SF4</td>
<td>16.1</td>
<td>10.6</td>
</tr>
<tr>
<td>SF6</td>
<td>14.4</td>
<td>10.4</td>
</tr>
<tr>
<td>SSF1</td>
<td>20.8</td>
<td>8.5</td>
</tr>
<tr>
<td>SSF3</td>
<td>17.2</td>
<td>8.7</td>
</tr>
<tr>
<td>SSF5</td>
<td>19.2</td>
<td>10.3</td>
</tr>
</tbody>
</table>
TABLE 4. Percent recovery of Li and \textit{E. coli} NAR from surface flow wetlands 2, 4, and 6, and subsurface flow wetlands 1, 3, and 5 during summer and winter tracer studies

<table>
<thead>
<tr>
<th>Surface flow</th>
<th>Summer</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF2</td>
<td>145</td>
<td>79</td>
</tr>
<tr>
<td>SF4</td>
<td>83</td>
<td>69</td>
</tr>
<tr>
<td>SF6</td>
<td>92</td>
<td>50</td>
</tr>
<tr>
<td>Subsurface flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSF1</td>
<td>18</td>
<td>106</td>
</tr>
<tr>
<td>SSF3</td>
<td>31</td>
<td>76</td>
</tr>
<tr>
<td>SSF5</td>
<td>11</td>
<td>85</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percent of \textit{E. coli} NAR recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface flow</td>
</tr>
<tr>
<td>SF2</td>
</tr>
<tr>
<td>SF4</td>
</tr>
<tr>
<td>SF6</td>
</tr>
<tr>
<td>Subsurface flow</td>
</tr>
<tr>
<td>SSF1</td>
</tr>
<tr>
<td>SSF3</td>
</tr>
<tr>
<td>SSF5</td>
</tr>
</tbody>
</table>

The relationship between wetland types is not so obvious within the winter tracer study data. \textit{E. coli} NAR effluent concentrations peaked within the SF and SSF wetlands at approximately the same time during the winter study (Fig. 2). SSF wetlands 1, 3, and 5 peaked on days 4, 4, and 6 at 36,000, 72,000, and 86,000 CFU per 100 mL, respectively. SF wetlands 2, 4, and 6 peaked on days 4, 4, and 5 at 112,000, 36,000, and 190,000 CFU per 100 mL, respectively. In winter, both SF and SSF wetlands showed higher peak \textit{E. coli} NAR effluent concentrations than during the summer study. Inactivation of \textit{E. coli} NAR proceeds according to first-order kinetics, which decrease with lower temperatures (Schnoor 1996). This may explain the elevated \textit{E. coli} NAR effluent concentrations observed during the winter study; winter temperatures were 10 to 15°C lower than summer temperatures (Fig. 2). Earlier and elevated peaks suggest the SF and SSF wetlands experienced reduced retention times during the winter study. Average outflow volumes from the SF and SSF wetlands were similar during the summer and winter tracer studies (Table 1). The actual volume available for flow through the wetlands may have been reduced due to accumulation of solids within the wetlands. Earlier and elevated peaks would be expected because a reduced volume within both the SF and SSF wetlands would shorten the hydraulic retention time.

\textit{E. coli} NAR effluent concentrations peaked before Li concentrations within wetlands 1, 3, 4, and 5 during the summer tracer study, and within wetlands 1, 2, 3, and 4 during the winter tracer study. \textit{E. coli} can be motile, propelled by rotating flagella, are able to sense attractants and repellents, and move to a more favourable environment by chemotaxis (Sourjik 2004). It is possible that \textit{E. coli} NAR were motile within the wetlands and moved more quickly than Li towards the outlet, hence the earlier peak concentrations. This presents a complex challenge for wetland design and modelling bacteria transport.

The percent of \textit{E. coli} NAR tracer that was recovered during the summer and winter studies was very low (Table 4). \textit{E. coli} NAR losses may be attributed to the wetland’s removal mechanisms that can include natural die-off, or adsorption of bacteria to solid particles which then become trapped or settle from the water column. During both the summer and winter studies, the percent recovery was higher within the SF wetlands compared with the SSF wetlands (Table 4). This calculation supports the observations discussed previously. The SF wetlands were more efficient for removing bacteria because they experienced longer retention times and likely trapped more solid particles compared with the SF wetlands. A higher percentage of the biotracer was recovered during the winter study than during the summer. Increased recovery during the winter may be due to reduced inactivation because of colder temperatures, shorter retention times, and short-circuiting.

Utilizing different enumeration techniques for \textit{E. coli} NAR during the winter and summer tracer studies may also be a source of discrepancy within this data, however, differences should be minor because the mTEC and m-ColiBlue24 plates produced similar counts when compared in a lab test.

Lithium

The summer Li data distribution [Fig. 3(a)] is similar to the \textit{E. coli} NAR data distribution [Fig. 2(a)]. Lithium effluent concentrations peaked earlier and higher within the SF wetlands during the summer study. Li was first detected on days 1 and 2 in the SF wetland effluent during the summer study. Li concentrations peaked in SF wetlands 2, 4, and 6 on days 9, 11, and 11 at 0.527 mg-L⁻¹, 0.325 mg-L⁻¹, and 0.323 mg-L⁻¹, respectively. Although these peak Li concentrations occurred on days 9 and 11 within the SF wetlands, the peaks were spread out [see Fig. 3(a)]. Li concentrations within the effluent from SF wetlands 2, 4, and 6 reached 0.46 mg-L⁻¹, 0.2 mg-L⁻¹, and 0.3 mg-L⁻¹ on days 2, 5, and 3, respectively. These wide peaks within the SF wetlands during the summer study indicate substantial mixing. Li effluent concentrations peaked lower and later within the SSF wetlands during the summer study. SSF wetlands 1, 3, and 5 peaked at days 17, 13, and 13 at 0.168 mg-L⁻¹, 0.199 mg-L⁻¹, and 0.076 mg-L⁻¹, respectively. The SSF wetlands experienced more pronounced peaks than the SF wetlands during the summer study. Li was first detected in SSF wetland effluent on days 11 and 12. This suggests that the hydraulic retention time was shorter within the SF wetlands than the SSF wetlands during the summer tracer study. This observation is consistent with the \textit{E. coli} NAR data from the summer tracer study.

Again, the Li data from the winter study is similar...
to the E. coli NAR observed data (Fig. 3). SF wetlands 2, 4, and 6 peaked on days 4, 6, and 5 at 1.125 mg·L⁻¹, 0.703 mg·L⁻¹, and 1.19 mg·L⁻¹, respectively. SSF wetlands 1, 3, and 5 peaked on days 6, 5, and 6 at 2.06 mg·L⁻¹, 1.47 mg·L⁻¹, and 1.38 mg·L⁻¹, respectively. All wetlands (SF and SSF) showed peak Li effluent concentrations at approximately the same time during the winter study. Peak Li concentrations are much higher than those observed during the summer study. As discussed previously in the E. coli NAR section of this report, these elevated peak concentrations can be attributed to channelling and/or a reduced hydraulic retention time. Li effluent concentrations were slightly higher within the SSF wetlands than the SF wetlands during the winter study. This may indicate that the SSF wetlands experienced more channelling than the SF wetlands. This is possible since the accumulation of solids within the SSF wetlands would have more influence on flow patterns than within the SF wetlands. The accumulation of solids within the SSF wetlands would fill void spaces that are necessary for treatment and distribution of flow. If void spaces become clogged with solids, the wastewater flow may become restricted and a preferential path could form. Channelling or short-circuiting may have caused the elevated peaks and shorter retention times during the winter tracer study. Rash and Liehr (1999) found that short-circuiting is common in SSF wetlands.

The mass recovery associated with Li was higher within the SF wetlands during the summer study. During the summer study, 83 to 145% of Li was recovered from the SF wetlands, while only 11 to 31% was recovered from the SSF wetlands (Table 4). Li concentrations were assumed to be constant with flow throughout the day, and concentrations were assumed to follow a straight line between data points. This simple estimation procedure may explain the percent mass recovery of 144 that was calculated for SF wetland 2 (Table 4). The large amount of Li that was lost within the SSF wetlands during the summer study may have been caused by Li adsorbing to organic matter. The crushed rock and pea stone could have provided space for trapping organic material and Li particles. A higher percentage of Li was recovered within the SF wetlands during the winter study; this supports the idea that the SSF wetlands experienced shorter retention times due to short-circuiting. Adsorption sites within the SSF wetlands were likely exhausted and shorter retention times would have increased Li recovery during the winter study. Percent recovery decreased slightly within the SF wetlands during the winter study. Accumulating solids within the SF wetlands may have created additional adsorption sites for Li particles during the winter study.

**Mean residence time, t_m.** In Table 3, the calculated mean residence time for each wetland during the summer and winter studies are presented. The results support the observations and discussion regarding the E. coli NAR and Li RTD functions. The SSF wetlands experienced longer retention times than the SF wetlands during the summer study. The mean residence time for the SF wetlands averaged 14 days, while the SSF wetlands averaged 19 days. The lower E. coli NAR effluent concentrations within the SSF wetlands can be attributed to longer retention times and therefore increased die-off and/or removal.

The mean residence times calculated for the winter study were not as representative of wetland type when compared with the summer study. The average retention time for the SSF wetlands was 9.9 days, while the SF wetlands averaged 9.2 days. These calculations also agree with the observations discussed previously in the E. coli NAR and Li sections. The mean residence times for the SF and SSF wetlands were approximately the same. This may be caused by a reduction in wetland volume due to cattail growth and/or accumulation of solids. The elevated E. coli NAR and Li effluent concentrations observed during the winter tracer study can be attributed to the shorter retention times. ET rates did not likely have a significant effect on the RTD functions because outflow volumes were similar between the summer and winter studies (Table 1).

**Dispersion coefficient, D.** Dispersion is a measure of the rate of mass transport due to mixing within a waterbody (Schnoor 1996). In Table 3, the longitudinal dispersion coefficients that were calculated for each of the SF and SSF wetlands (based on the summer and winter tracer study data) are shown. The dispersion coefficients ranged from 1.3x10⁻⁶ to 4.2x10⁻⁶ m²·s⁻¹ for the SF and SSF wetlands during the summer tracer study. Dispersion was slightly higher within the SF wetlands during both the summer and winter studies. During the winter tracer study, dispersion coefficients ranged from 8.7x10⁻⁷ to 2.3x10⁻⁶ m²·s⁻¹. This range of dispersion coefficients is typical for the vertical transfer of particles within lakes (Schnoor 1996). These nonideal hydraulic characteristics indicate that dispersion is an important transport process within SF and SSF wetlands. Mass transport within rivers is driven by advection, or the movement of water (Schnoor 1996). Advective velocity within treatment wetlands is relatively low; low flow conditions within the wetlands may explain the dispersive properties of the system. Many wetland models consider advective transport only: for example, the plug flow model (Kadlec and Knight 1996). Purely advective models should be considered inaccurate for modelling mass transport through low flow treatment wetlands.

**Conclusions**

Difco mTEC agar was found to be better than m-ColiBlue24 for enumerating E. coli NAR because total coliform growth was eliminated and colonies were more visible with the mTEC.

Dispersion is an important mass transport process in both SF and SSF treatment wetlands. Dispersion coefficients were slightly higher within the SF wetlands
during the summer and winter studies. When modelling or designing treatment wetland systems, dispersion processes should be considered important.

Bacteria removal within a treatment wetland system may be attributed to natural inactivation or adsorption to solid particles, which can then become trapped or settle from the water column. SSF treatment wetlands may achieve more efficient removal of bacteria during the first few months of operation compared with the SF wetlands. Long-term operation of SF and SSF treatment wetlands may cause a reduction in wetland volume due to the settling of solids or clogging of pore spaces. This can cause reduced retention times and create channelling or short-circuiting, which will reduce treatment efficiency. SSF wetlands may be more susceptible to channelling or short-circuiting due to the accumulation of solids within pore spaces.

Modelling bacteria transport through treatment wetlands presents a complex challenge because *E. coli* may be motile within wetlands. Winter conditions may also reduce SF and SSF treatment wetland efficiency because of reduced inactivation of bacteria in colder temperatures.

Additional studies should be performed to further investigate the impacts of wetland type and maturity, and climate conditions on treatment wetland efficiency.

**Acknowledgments**

This research is funded by the Canadian Water Network (CWN). We would also like to thank Matthew Murphy and Andy Vanderzaag for their help with sampling and data collection.

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Received: 27 February 2007; accepted: 19 June 2008.
Water Quality Assessment in the Application of Stormwater Reuse for Irrigating Public Lands

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Stormwater reuse for irrigating public lands presents a viable option for reducing potable water demand in urban settings. However, stormwater generally contains high pollutant levels, which may cause adverse effects on public health and the environment. Water quality in a stormwater retention pond in the City of Calgary, Alberta, was examined in order to assess the feasibility of reusing stormwater for irrigation purposes. Field campaigns were conducted in the 2004, 2005, and 2006 irrigation seasons. The water quality data indicated that the pond water quality generally satisfies the requirements for stormwater recycled as irrigation water. Relationships between stormwater quality and climatological variables were investigated using correlation and regression analysis. Their correlations suggest that intermittent rain events contribute to elevated microbial levels and total suspended solids (TSS). Other climatological variables—air temperature, cloud cover, wind speed, and relative humidity—are also correlated with certain water quality parameters including fecal coliform (FC), TSS, nutrients, and conductivity. Formulated regression equations demonstrate good predictions of observed FC and TSS using climatological variables. Results showing stormwater quality as a function of climatological variables imply that climate change might have potential influence on stormwater quality.

Key words: water quality, stormwater reuse, stormwater pond, climate change, irrigation water

Introduction

Canada, on the whole, has relatively abundant water resources. As a result, water reuse is currently practised infrequently in Canada (Exall et al. 2004). However, the water supplies in the Canadian Prairies are generally scarce due to their semiarid climate. This region has been struggling to formulate new strategies that exploit alternative sources of water to safeguard water supplies against increasing potable water demand resulting from rapid population growth and economic development. Moreover, recent studies on climate change in this region and the resulting consequences (Toyra et al. 2005) have escalated concerns for the availability of water resources. Gan (1998) statistically analyzed historical data and indicated that the Canadian Prairies have become warmer and somewhat drier in the last four to five decades, and that Prairie droughts will continue to occur. Climate change reinforces the need for adapting to changing conditions, despite the uncertainty involved in these climatic changes (Gan 2000).

Water conservation has been considered an effective means to extend available water resources, particularly in urban settings. Besides various conservation strategies, water reuse as an alternative water supply presents another attractive option (Crook and Surampalli 1996). One of the challenges in urban water management is to further develop methods for recycling and using stormwater in applications that do not require high water quality such as toilet flushing and irrigation of parks and local agriculture (Niemczynowicz 1999). Urban stormwater runoff has been identified as one of the significant causes of water quality impairment in water receiving bodies (Tsihrintzis and Hamid 1997). Stormwater runoff from streets may be highly polluted and render stormwater reuse applications unfeasible (Exall 2004). In particular, the transmission of pathogenic microorganisms through irrigation may result in pathophysiological conditions ranging from gastrointestinal and upper respiratory illness, to skin irritations, and/or eye, ear, and wound infections (House et al. 1993). Microorganisms are therefore a major concern in the environmental assessment of stormwater reuse for irrigating public lands because the stormwater application may expose the public to potentially microbially contaminated water.

More recently, water quality issues have attracted increasing attention and concern, and a large number of studies have focused on stormwater quality and pollutant removal in stormwater ponds. Stormwater ponds have been shown to be effective facilities for mitigating the impacts of urban runoff (Mallin et al. 2002). The ponds are primarily designed to reduce solid constituents such as TSS and pollutants associated with suspended solids by settling. However, it has been shown that wet ponds can effectively remove other pollutants such as nutrients, microorganisms, and some metals (Davies and Bavor 2000). On the other hand, climate change is believed to

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contribute to changes both in water quantity and in water quality. The effect on water quality, however, is poorly understood (Rose et al. 2000). Changes in temperature, precipitation/rainfall, and solar radiation etc., are expected due to global warming (IPCC 2001). Murdoch et al. (2000) stated that changes in climate (precipitation and temperature) can have a significant effect on the quality of surface waters. The response of stormwater quality to climatological variables may be used to infer potential changes in stormwater quality resulting from a changing climate.

The City of Calgary in Alberta has initiated a program to irrigate public lands using stormwater detained in the Inverness Stormwater Pond located in the southeastern quadrant of the city. The intention of the measure is to reduce pressures on water supplies over a long-term time frame. A study of perceptions and attitudes towards recycling stormwater for irrigation by Hwang et al. (2006) conducted at the site indicated that the public’s greatest concerns over this stormwater application involves water quality. The twofold purpose of this paper is to conduct an overall technical assessment of the level of water quality of the stormwater available for reuse as irrigation water, and to investigate potential relationships between stormwater quality and climatological variables. The results are based on three water quality sampling campaigns carried out in the 2004, 2005, and 2006 irrigation seasons.

Materials and Methods

Study Site

The Inverness Stormwater Pond is adjacent to Inverness Park located in southeast Calgary. The park consists of two components: a linear park with a walking trail around the pond, and a high use area with a playground and sports fields. The current intention of the City is to irrigate the linear park with stormwater, while the high use area is irrigated with potable water. The catchment draining into the pond is approximately 150 ha and consists of two developed residential subdivisions and one subarea under residential development. Stripping and grading operations on the land commenced in 2004 and was completed in 2007. Approximately 50% of the area drains into the pond via overland flow. Erosion and sediment control measures, such as silt fencing, v-ditching and temporary ponding for controlling runoff, etc. were in place during the study period. The stormwater drainage system has been designed based on the dual drainage concept, namely the minor system and the major system. During frequently occurring, low intensity rain events, storm runoff drains into the pond through underground storm sewers from the developed subareas. During rain events that exceed the design capacity (5 year return period storm) of the underground storm sewer system, stormwater may also run into the pond through overland
flow paths. The pond was constructed to include a permanent water pool (permanent water level [PWL] = 1,028.8 m) with a depth of 3.8 m (approximately 170,000 m$^3$) and an active storage capacity of 95,000 m$^3$. There are six inlets and one outlet around this pond as shown in Fig. 1. The pond outlet, which houses an outflow weir (its crest is equal to the PWL), is located in the northwest corner of this pond. Outflow occurs when the pond water level exceeds the PWL. The estimated average annual runoff (based on the normal annual rainfall amount) is 154,000 m$^3$. The time needed to drain the pond down depends on the stormwater runoff volume and pond water level prior to each event. Irrigating the linear park involves withdrawals from the pond through a pump-pipe system located near grab site 1. The city irrigates the area in the early morning in order to minimize the potential risk of public exposure to contaminated stormwater because of this new strategy. The irrigation system is controlled by devices (rain switch and freeze switch) that prevent irrigation during rain or freezing conditions. Once the washer of the rain switch accumulates a set amount of rainfall (an adjustable collar is set to 6 mm in Calgary), the device is activated to shut off the irrigation system. Watering recommences until the washer dries out, which typically takes a couple of days. The normal overall irrigation demand is approximately 15,700 m$^3$ in an irrigation season.

This study site is located in a semiarid region. The normal annual rainfall amount at the Calgary International Airport (1971 to 2000) is 320.6 mm (Environment Canada 2007). Rainfall events generally occur from May to September, while the average daily temperature is roughly 10ºC or greater in these months.

**Table 1. Climatic conditions**

<table>
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<tr>
<th>Month</th>
<th>June</th>
<th>July</th>
<th>August</th>
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</tr>
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<tr>
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<td>16.2</td>
<td>15.6</td>
<td>10.8</td>
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<td></td>
</tr>
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<td>9</td>
<td>10</td>
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</table>

*Data from Environment Canada (2007).*

Irrigation generally begins at the end of June and terminates at the end of September. Table 1 shows the monthly normal climatic conditions and meteorological conditions of the three irrigation seasons, respectively. The summer of 2004 was quite dry with frequently occurring, small rainfall events. The same periods for 2005 and 2006 were comparatively wet and several heavy rainfall events occurred during these periods.

**Water Quality Concerns**

The primary source of water to the pond is stormwater runoff generated from intermittent rainfall events. Stormwater runoff transports various nonpoint source pollutants into the pond, including eroded solids, nutrients, oxygen demanding compounds, and microorganisms etc., which accumulate in the watershed prior to the event. Stormwater is generally known to degrade the physical quality of the receiving water body as well as the chemical quality (by enhancing nutrient levels) and bacteriological quality (Bannerman et al. 1993). TSS influences the appearance of the aquatic environment, benthic communities, and fish habitat. Levels of nutrients are a major concern in standing water bodies like ponds, lakes, and estuaries. The major concern in reusing stormwater for irrigation is public health. Lazarova and Bahri (2005) stated that trace elements are generally not a problem for irrigation with recycled water. However, they also pointed out that the metals B, Cd, Cu, Cr, Ni, Zn, Se, Mo, and Pb are considered to be an environmental concern, and that these metals may cause human health effects because they can be transferred to animals or humans through various pathways depending on their
concentrations. Davis et al. (2005) observed strong seasonal differences in indicator bacteria concentrations in Canyon Lake, California. High concentrations of fecal coliform were found in the summer season and low levels in the cooler winter months. It has also been well documented that microbiologically contaminated stormwater may cause water-borne disease outbreaks (Hrudey et al. 2003). Microbial quality has been a crucial factor in determining the safety of drinking, recreation, and irrigation waters. Consequently, it should be considered a vital aspect in examining the use of stormwater for irrigation, because of the significant potential adverse effects on public health. In this study area, the source of microorganisms comes primarily from animals and wild birds’ excreta.

Water quality standards commonly use microbial indicators of fecal contamination to regulate water microbiological quality for surface water uses. Pathogenic microorganisms including bacteria, protozoa, and viruses are real concerns in fecal contaminated water. These indicators represent a potential risk to public health if the fecal contaminated water is consumed or contacted by the public directly or indirectly. However, it is well understood that there are limitations and disadvantages to using these indicators to predict the presence of pathogens (National Research Council of the National Academies 2004) and to evaluate microbial risk. Past studies on waterborne protozoa of serious public health concerns have focused on the occurrence of Cryptosporidium and Giardia (Roach et al. 1993). Moe (2002) pointed out that water contamination by both human and animal feces is an important mode of transmission for Cryptosporidium and Giardia. Moreover, he stated that the incidence in humans due to enteric bacteria, Escherichia coli O157:H7, has increased and threatens to increase in the future. The prevalence of E. coli O157:H7 and Salmonella was investigated in surface waters of southern Alberta (Johnson et al. 2003). Therefore, testing for Cryptosporidium, Giardia, E. coli O157:H7, and Salmonella were also carried out in this study.

Sample Collection and Analyses

A stormwater quality sampling campaign was conducted during the 2004, 2005, and 2006 irrigation seasons from June through September/October. Three types of water samples were collected: “surface grab samples,” “irrigation water samples” (water taken from the pond through the pump-pipe irrigation system), and “surface water samples” that were specifically for assaying pathogens. All sampling was conducted with no attempt made to favour either rain events or dry periods. Surface grab samples were taken at 6 grab sampling sites (Fig. 1). These samples were collected once a week in the morning, and occasionally twice a week at a depth of roughly 10 to 20 cm below the water surface using a plastic container. They then were transferred to sterile plastic bottles (approximately 2 L per sample).

Irrigation water samples were taken from the pump wet well using a Sigma Portable Sampler. The inlet pipe of the well was situated at a depth of 1.0 m below the PWL. The irrigation cycle began at 12:05 a.m. and went to 7:30 a.m. in order to minimize the potential health risk from exposing the public to potentially contaminated water during irrigation. Three irrigation water samples (2 L per sample) were collected at 1:00, 2:45, and 4:30 a.m. weekly.

Weekly surface water samples in 2004 and monthly surface water samples in 2005 and 2006 were collected at grab sampling site #1 (at a 10- to 20-cm water depth) for analyzing pathogens. An approximately 500 mL surface water sample was collected using a peristaltic pump. The sample was filtered through fi ltra-max filters on site and then delivered in a sealed filter housing. The collected sample was tested for the presence/absence of E. coli O157:H7 and Salmonella. In addition, a 50-L surface water sample was collected from the same location for measuring Cryptosporidium and Giardia.

Water temperature, conductivity, pH, dissolved oxygen (DO), and turbidity were measured using a YSI 550, YSI 30, Accumet 62 pH Meter, and a LaMotte 2020 Turbidity Meter at each grab site when surface grab samples were collected. The collected surface grab samples and the irrigation water samples were transported to laboratories where they were immediately prepared and analyzed. Analyses of PO₄³⁻, NH₃-N, NO₃-N, 5-day biochemical oxygen demand (BOD₅), and TSS from surface grab samples were carried out at the Civil Engineering Wastewater Lab at the University of Calgary. Microbial indicators—total coliform (TC), fecal coliform (FC), and E. coli—from all surface grab samples and irrigation water samples were assayed using a membrane filter method according to Standard Methods (APHA 1998) at the Provincial Laboratory for Public Health in Calgary. These methods report analysis results as colony-forming units (cfu) per 100 mL of water. The tests on pathogens were also conducted at the Provincial Laboratory. Concentrations of various metals from irrigation water samples were measured using inductively coupled plasma mass spectrometry (ICP-MS) at the City of Calgary’s Bonnybrook Laboratory.

Results and Discussion

Quality Level of Pond Water

There is no water quality guideline specific to irrigation water used for public lands. In order to investigate the applicability of stormwater reuse for irrigating parklands, two guidelines—Surface Water Quality Guidelines for Use in Alberta (Alberta Environment 1999) and Guidelines for Canadian Recreational Water Quality (Health and Welfare Canada 1992)—were consulted to provide some measure of maximum limits for various water quality parameters. Previous studies conducted at this study site
(Hwang et al. 2006) indicated that the park was being used predominantly for strolling, sitting, and walking dogs, and that many users were elderly or very young children who are most susceptible to waterborne illnesses. People who visit the area might accidentally ingest pollutants directly or indirectly through hand-to-mouth activities. Thus, the portion of the Alberta guidelines dealing with agricultural practices was used.

Although TC is also mentioned in these guidelines, FC and *E. coli* are better microbial indicators for predicting the presence of fecal contamination than TC. For this reason, FC and *E. coli* were used in the water quality assessment. The concentration of FC and *E. coli* determined from the geometric mean of not less than five samples taken over not more than a 30-day period should not exceed 200 organisms per 100 mL, nor exceed these numbers in more than 20 percent of the samples examined during any month, according to the guidelines for recreational purposes. In addition, the Canadian guideline documented that the probability for the presence of *Salmonella* is high when FC density exceeds 200/100 mL. Therefore, the concentrations of FC and *E. coli* should not exceed the (maximum) limits of 200/100 mL. If concentrations under these limits are observed, it is believed that the presence of pathogens (such as *Salmonella*) may occur but at very low frequencies.

SAR, or sodium adsorption ratio, is a parameter used to indicate the permeability of soil to water and air, which indicates the potentially long-term impact of recycled water on soil. There is no restriction on the use of recycled water for irrigation if the value of SAR is less than 3, but severe damage can occur when SAR is over 9 (Lazarova and Bahri 2005).

Table 2 gives ranges of microbial indicators and SAR assayed from irrigation water samples in the three sampling seasons, respectively. The maximum concentrations of metals mentioned above are listed in Table 3 for each year. The metals Cd, Cr, and Mo, which are not shown in this table, were all below detection limits in all samples during the three seasons. The results capture the quality of stormwater available for irrigating the park. The irrigation water samples taken after a heavy rain event on September 11 in the 2005 irrigation season exceeded the allowed maximum density of microbial indicators; however, irrigation is not performed in wet conditions. The maximum SAR of 6.1 was found from the sample collected on August 2, 2006. A very high sodium concentration of 274 mg/L was observed from the sample. However, sodium concentrations for all other samples were below 100 mg/L during the three irrigation seasons.

Under dry periods and low flow conditions, the concentrations of these water quality parameters were generally below guideline values. Moreover, no pathogens were detected from surface water samples during the entire water quality monitoring program (data not shown). These results indicate that stormwater quality in this pond generally meets guideline requirements. Thus, stormwater in the pond is a suitable alternative to potable water for irrigating public lands based on observations in the three irrigation seasons.

Figure 2 demonstrates the observed averaged microbiological quality (both FC and *E. coli*) level for both irrigation water samples and surface grab samples for each irrigation season in box and whisker plots. The box covers the interquartile range; its bottom is the 25th percentile and its top is the 75th percentile; whiskers extend from the box to the furthest datum. Most irrigation water samples and surface grab samples were collected on the same days, but there was a one- or two-day lag between them in a few samples. A significant difference (*p* < 0.05) between them was only detected in the 2004 irrigation season by using the nonparametric Kruskal-Wallis test. Higher concentrations of microorganisms were observed from surface grab samples. Microbial concentrations in irrigation water samples were under

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC (cfu/100 mL)</td>
<td>72</td>
<td>1</td>
<td>37 (350a)</td>
<td>2</td>
</tr>
<tr>
<td><em>E. coli</em> (cfu/100 mL)</td>
<td>68</td>
<td>1</td>
<td>37 (260a)</td>
<td>1</td>
</tr>
<tr>
<td>SAR</td>
<td>1.9</td>
<td>1.7</td>
<td>1.7</td>
<td>0.7</td>
</tr>
</tbody>
</table>

*Sample collected just after a heavy rainfall event on September 11, 2005. Very high concentration of sodium (274 mg/L) from the sample taken on August 02, 2006. Occurrence of severe damage.*

**Table 3. Maximum concentrations of concerned metals in irrigation water samples**

<table>
<thead>
<tr>
<th>Metal</th>
<th>Maximum concentration</th>
<th>Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2004</td>
<td>2005</td>
</tr>
<tr>
<td>B (mg/L)</td>
<td>0.0582</td>
<td>0.0625</td>
</tr>
<tr>
<td>Cu (mg/L)</td>
<td>LDL</td>
<td>LDL</td>
</tr>
<tr>
<td>Ni (mg/L)</td>
<td>0.0138</td>
<td>0.034</td>
</tr>
<tr>
<td>Zn (mg/L)</td>
<td>0.036</td>
<td>0.0659</td>
</tr>
<tr>
<td>Se (mg/L)</td>
<td>LDL</td>
<td>0.0029</td>
</tr>
<tr>
<td>Pb (mg/L)</td>
<td>0.0083</td>
<td>0.0182</td>
</tr>
</tbody>
</table>

*LDL: less than detection limit. Relying on soil pH value.*

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the allowable densities even when surface grab samples exceeded the guideline values during this season. Spatial differences among the grab sites were also found during the season. The authors speculate that pollutants from other sources were entering into the pond temporally during this time period. Differing from the 2004 season results, the surface grab sample results from the 2006 and 2005 irrigation seasons are felt to represent the microbiological quality of irrigation water samples.

Correlations of other water quality parameters with climatological variables were also calculated based on observations in surface grab samples. Available climatological variables—air temperature, cloud cover, wind speed, rainfall, and relative humidity (RH)—were utilized in the analysis. The rainfall data were obtained from the City of Calgary (rain gauge #26 located in McKenzie Towne); air temperature, cloud cover, wind speed, and RH were obtained from the weather station at the Calgary International Airport.

The correlations between averaged water quality parameters for all grab sites and climatological variables were investigated in the three sampling years. The correlation coefficients were computed using totals (rainfall depth) and averages (temperature, cloud cover, wind speed, and RH) observed on the sampling day (1-day), as well as over the sampling day plus 1, or 2, 3, 4, 5, or 6 days preceding the sampling day. Thus, correlation coefficients were computed based on 1 day (the sampling day), or 2, 3, 4, 5, 6, or 7 day periods. The highest Pearson correlation coefficients (statistical significance $p < 0.10$) for all climatological variables tested are shown in Table 4. Not surprisingly, the microbial indicators, TC, FC, and $E. coli$, are highly related to each other; and a high correlation between TSS and turbidity ($p < 0.001$) was also found in this study (as shown in Table 7). Thus correlation coefficients of FC and TSS with climatic variables were presented. Although the results did not give consistent correlations between water quality and climatological variables during the three sampling seasons, they did show the potential relationships of stormwater quality with climatological variables. For instance, some pollutant constituents, such as FC, DO, conductivity, and BOD$_5$, are primarily related to climatological variables over short time periods, but other quality parameters such as TSS and nutrients are primarily correlated with variables in a cumulative manner. These results indicate that the effect of climatological variables on pollutants is variable.

Figure 3a and 3b show the time series of FC and the climatic variables which were identified to be correlated with this quality parameter in the 2005 and 2006 seasons, respectively. Note that the time series of FC for the 2004 season is not shown because they were suspected to be influenced by other pollutant resources. These results demonstrate that the intermittent rain events are one of major causes of elevated microorganisms in the stormwater pond. They also illustrate that the magnitude of FC in the pond water follows the variability in rainfall depth. Although irrigation was not required during raining or wet conditions, the risk may theoretically exist as it cannot be ruled out. However, that risk, in this particular case, appears to be quite small. Moreover, the correlations indicate that the microbiological quality level is dependant on climatic variables, such as cloud cover, wind speed, and RH, under dry periods; however no significant correlation was found between FC and air temperature in all these irrigation seasons. These

**Water Quality and Climatological Variables**

A correlation analysis was conducted in order to investigate relationships between stormwater quality and climatological variables, which may be suggestive of the potential consequences of a changing climate, such as changes in temperature and rainfall, etc., on stormwater quality. Because it was observed that the microbiological quality of averaged surface grab samples generally represent that of irrigation water samples, the correlation between microbial quality and climatological variables was only performed on surface grab samples.

Correlations of other water quality parameters with climatological variables were also calculated based on observations in surface grab samples. Available climatological variables—air temperature, cloud cover, wind speed, rainfall, and relative humidity (RH)—were utilized in the analysis. The rainfall data were obtained from the City of Calgary (rain gauge #26 located in McKenzie Towne); air temperature, cloud cover, wind speed, and RH were obtained from the weather station at the Calgary International Airport.

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### Table 4. Pearson correlation coefficients (statistical significance $p < 0.10$) between water quality parameters and climatic variables.a,b

<table>
<thead>
<tr>
<th>Parameters</th>
<th>1-day</th>
<th>2-day</th>
<th>3-day</th>
<th>4-day</th>
<th>5-day</th>
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a Cond: conductivity; Temp: water temperature.

b Sample sizes in the 2004, 2005, and 2006 seasons are 15, 18, and 14, respectively.

c Correlation coefficient in 2004.

d Correlation coefficient in 2005.

e Correlation coefficient in 2006.
relationships illustrate that these climatic variables could have significant impacts on the growth and mortality of microorganisms in water bodies between rain events.

High correlations were observed between TSS and the investigated climatological variables in the 2004 and 2006 irrigation seasons, while no relationships between them were detected in 2005. As shown in Fig. 4, the observed high variability in TSS among the grab sampling sites, and the particularly high TSS at site #2 and site #6 (which are close to the area that is under development) at the beginning of the 2005 season, could be the result of the active construction during the time period, which consequently influences the relationship between TSS and climatic variables. The time series of TSS and various climatic variables are presented in Fig. 5 for the 2004 and 2006 seasons, respectively. The energy generated from both raindrops and runoff will transport solids into receiving waters, especially in areas where significant subsoil is exposed. In the 2004 irrigation season, the results show that TSS increased with time until the middle of September. There were small, frequently occurring rain events during the season, which appeared to have an insignificant effect on TSS. Air temperature, cloud cover, and wind speed were found to be significantly correlated with TSS and therefore influenced TSS concentrations in the 2004 season. The dry and warm climatological conditions coupled with the relatively motionless water in this pond (no single large stormwater runoff event flowed into and out of the pond in this period) might provide ideal conditions for stimulating excessive growth of organic matter (e.g., algae, plankton) in the 2004 growth season. This could result in increased TSS concentrations with time. However, it was observed that elevated TSS concentrations during the 2006 season were mainly attributed to rainfall events. Besides rainfall, significant correlations were also identified with air temperature, cloud cover, wind speed, and RH in this 2006 season. Between 2004 and 2006, opposite correlations between TSS and wind speed were detected.

As the correlation analysis shows, some stormwater quality parameters were correlated with climatological variables. Thus, multiple linear regression analysis was employed to further discern the influence of meteorology on FC and TSS. In these multiple regression models, the water quality variables were the dependents, while the climatological variables were the independents. The independent variables were selected based on a literature review and results from the correlation analysis.

Stormwater runoff undoubtedly washes off various nonpoint pollutants into receiving waters. Rainfall amounts along with other rainfall characteristics were used to develop multiple linear regression models for storm runoff volumes and pollutant loads and concentrations in the study by Brezonik and Stadelmann (2002).

Shallow water bodies are susceptible to wind-induced hydrodynamic disturbances. The disturbances can induce sediment resuspension, which in turn cause internal
pollutant loadings to the water column from bottom sediments (Kristensen et al. 1992). Wave characteristics (height, period, and wavelength) calculated from wind speed and fetch information were utilized in the model which predicted TSS in the water column due to sediment resuspension (James et al. 2004).

The surrounding environment has an influence on the mortality of coliform bacteria in aquatic systems. Bacterial cells are known to suffer injury from exposure to irradiance. Temperature is also expected to play a role in the decay rate of coliform bacteria. Studies by Auer and Niehaus (1993) and Canale et al. (1993) utilized irradiance and temperature together with the effect of sedimentation to model fecal coliform bacteria decay. However, no temperature influences on FC death rate were reported in the field observations by Auer and

Several studies have been conducted to develop models using environmental measurements to predict FC in various water bodies. Eleria and Vogel (2005) quantified the relationship between FC and climatological and hydrological variables for a river. Among various meteorological variables, rainfall and wind were shown to be essential factors in their models. Siewicki et al. (2007) used climatic factors including wind speed and rainfall along with characteristics of the watershed and physicochemical water quality parameters (pH, water temperature, and salinity) to construct a prediction model for FC in coastal ponds based on monthly sampling data. They found that both rainfall and wind lead to elevated FC concentrations.

Based on the literature survey, rainfall, temperature, radiation, and wind speed should be taken into consideration to develop regression equations. Measurements of radiation are not available for the City of Calgary. But previous studies (Kimura and Stephenson 1969) have demonstrated that cloud cover can be used to estimate solar radiation. Cloud cover essentially took the place of irradiance in the regression analysis. Along with these climatological variables, significant correlations between the water quality parameters and RH were found in this study; therefore, RH was also included in the analysis. All climatological variables which were found to be significantly correlated with water quality were used. However, temperature was not included in the regression analysis of FC because no significant correlation was identified between them. Data in 2004 and in 2005 were excluded when developing regression models for FC and TSS, respectively, because, as noted earlier, unexpected pollutant sources were believed to be influencing observations of FC in 2004 and TSS in 2005. The most statistically significant (lowest p-value) predictor was added into the models at each step until there were no predictors left. There were a total of 4 and 5 models for FC and TSS, respectively. In the case of water quality parameters that were found to correlate with climatological variables on different days (e.g., the highest correlation was found between TSS and wind2-day in the 2005 season, while the highest correlation was calculated between TSS and wind6-day in the 2006 season), the variable which had the lower p-value was chosen as the predictor and the other was excluded from the model. Results of all models are shown in Tables 5 and 6. All regression models for both FC and TSS were accepted at the 5% significance level. However, the value of $R^2$ for Model 2 in Table 5 showed a noticeable increase from Model 1’s $R^2$ value, but Models 3 and 4 did not increase the $R^2$ from Model 2 very much. This suggests that Model 2’s predictors of rainfall and wind are sufficient to predict FC. Similarly, Model 2 for TSS, which uses temperature and wind, produces the best predictions of TSS among the TSS models. In order to investigate the roles of all the noted climatological variables, regression coefficients and variance inflation factors of Model 4
He et al.

TABLE 5. Various regression models for FC*

Regression models between FC and climatological variables

<table>
<thead>
<tr>
<th>Model</th>
<th>$R^2$</th>
<th>Adjusted $R^2$</th>
<th>p-value</th>
<th>F-value</th>
<th>Table F-value (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.831</td>
<td>0.820</td>
<td>&lt;0.001</td>
<td>147.34</td>
<td>24.17</td>
</tr>
<tr>
<td>2</td>
<td>0.862</td>
<td>0.848</td>
<td>&lt;0.001</td>
<td>90.52</td>
<td>3.32</td>
</tr>
<tr>
<td>3</td>
<td>0.868</td>
<td>0.849</td>
<td>&lt;0.001</td>
<td>61.23</td>
<td>2.93</td>
</tr>
<tr>
<td>4</td>
<td>0.868</td>
<td>0.844</td>
<td>&lt;0.001</td>
<td>44.45</td>
<td>2.71</td>
</tr>
</tbody>
</table>

Regression coefficients for Model 4

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coefficient</th>
<th>p-value</th>
<th>$R^2$</th>
<th>Variance inflation factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rain$_{day}$ (mm)</td>
<td>3.48</td>
<td>&lt;0.001</td>
<td>0.360</td>
<td>1.56</td>
</tr>
<tr>
<td>Wind$_{day}$ (m/s)</td>
<td>-14.46</td>
<td>0.0239</td>
<td>0.315</td>
<td>1.46</td>
</tr>
<tr>
<td>RH$_{day}$ (%)</td>
<td>0.12</td>
<td>0.7749</td>
<td>0.500</td>
<td>2.00</td>
</tr>
<tr>
<td>Cloud$_{day}$ (10$^3$)</td>
<td>-2.29</td>
<td>0.2873</td>
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<tr>
<td>Interceptor</td>
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</tr>
</tbody>
</table>

*Sample size = 32.

Model 1: rain; Model 2: rain and wind; Model 3: rain, wind, and cloud cover; Model 4: rain, wind, cloud cover, and RH.

TABLE 6. Various regression models for TSS*

Regression models between FC and climatological variables

<table>
<thead>
<tr>
<th>Model</th>
<th>$R^2$</th>
<th>Adjusted $R^2$</th>
<th>p-value</th>
<th>F-value</th>
<th>Table F-value (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.49</td>
<td>0.448</td>
<td>&lt;0.001</td>
<td>23.08</td>
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<tr>
<td>2</td>
<td>0.586</td>
<td>0.533</td>
<td>&lt;0.001</td>
<td>16.31</td>
<td>3.4</td>
</tr>
<tr>
<td>3</td>
<td>0.602</td>
<td>0.529</td>
<td>&lt;0.001</td>
<td>11.07</td>
<td>3.03</td>
</tr>
<tr>
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<td>7.94</td>
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<tr>
<td>5</td>
<td>0.602</td>
<td>0.483</td>
<td>0.001</td>
<td>6.05</td>
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</table>

Regression coefficients for Model 5

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coefficient</th>
<th>p-value</th>
<th>$R^2$</th>
<th>Variance inflation factors</th>
</tr>
</thead>
<tbody>
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<td>Temperature$_{day}$ (°C)</td>
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<td>0.5352</td>
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<td>Wind$_{day}$ (m/s)</td>
<td>-4.66</td>
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<td>0.1977</td>
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<tr>
<td>RH$_{day}$ (%)</td>
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<td>0.524</td>
<td>0.5752</td>
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</tr>
<tr>
<td>Rain$_{day}$ (mm)</td>
<td>0.02</td>
<td>0.867</td>
<td>0.2325</td>
<td>1.3</td>
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<tr>
<td>Cloud$_{day}$ (10$^3$)</td>
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<td>Interceptor</td>
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</tbody>
</table>

*Sample size = 26.

Model 1: temperature; Model 2: temperature and wind; Model 3: temperature, wind, and RH; Model 4: temperature, wind, RH, and rain; Model 5: temperature, wind, RH, rain, and cloud cover.

for FC, and Model 5 for TSS, both of which include all the correlated climatological variables for the quality parameters, are listed in the Tables 5 and 6, respectively. Similarly, significant contributions of rain and wind on FC were identified, while temperature and wind were the most significant factors in the TSS model. The calculated and observed results for these two models are illustrated in Fig. 6.
Except for temperature, all predictor variables appeared to have similar (positive or negative) regression coefficients for both the FC and TSS models. Cloud cover, which from the literature is expected to have an influence on FC, was identified here to have negligible impacts on FC levels. Similarly, RH influences on FC and TSS were also negligible.

Rain was shown to be a significant cause of elevated FC concentrations. The role of rain on TSS was not as significant as that on FC levels. Although TSS was observed to be positively related to rain in the 2006 irrigation season, the influence of rain events on TSS could not be detected in the 2004 irrigation season (as shown in Table 4) which had frequent, small rain events. The sampling in these two seasons might not be able to readily detect the influence of storm events on TSS. The significant negative regression coefficients of wind in both FC and TSS models might suggest that the consequences of increases in FC and TSS at the surface water layer were not caused by sediment resuspension induced by wind. Zhu et al. (2007) also observed that wind-induced hydrodynamic disturbances have no influence on SS in the top water layer, although its influence was significantly shown in bottom layer in a large and shallow lake. Wakelin et al. (2003) stated that increases in TSS within stormwater retention basins in Winnipeg were probably caused by biomass accumulation from algal production based on their bi-weekly sampling. The negative regression coefficient for temperature in the TSS regression equation was somewhat expected because temperature was negatively correlated with TSS in the two seasons.

As Tables 5 and 6 show, calculated variance inflation factors were all greater than 1, indicating that some colinearities existed among the predictor variables. These factors however did not seem to cause serious multicolinearity problems in these models. A variance inflation factor greater than 10 is an indication of potential multicolinearity problems (Wetherill 1986). Quality data from three seasons were available for the analysis, but one season’s worth of data were excluded to construct the regression models. In addition, inconsistent correlations between wind speed and FC and TSS were found in the correlation analysis. Therefore, the influence of wind on FC and TSS should be further verified. Although the regression models appear to predict the observations fairly well, using the models in the regions (e.g., FC larger than 100 cfu/100 mL) where there are few data points may be questionable. The reliability of these regression models would be substantially improved by increasing the number of field studies and measurements.

In addition, Pearson correlation coefficients ($p < 0.10$) between averaged water quality parameters for all grab sites are also illustrated in Table 7 for the 2004, 2005, and 2006 irrigation seasons. In general, there were no consistent correlations between water quality parameters during the sampling time periods, except among microbial indicators and between TSS and turbidity. TC was highly correlated with FC in the 2004 ($r = 0.97$) and 2005 ($r = 0.95$) seasons, but their correlation was 0.66 in the 2006 season. Differing from FC, TC can also be found in the aquatic environment and in soil. Nonfecal coliform can grow at elevated temperatures (Baudisova 1997). However, elevated temperatures enhance die-off of fecal coliform bacteria as well (Flint 1987). A low ratio of TC and FC was found in July of 2006 when the water temperature was high. This may be the cause of the low correlation in the 2006 season. It was found that microorganisms are not highly related to TSS in both the 2005 and 2006 seasons, while a high correlation was found in the 2004 season. These results might be a consequence of observed annual temporal differences in these water quality parameters, such as microorganisms and TSS, in the three seasons. However, the correlation between TSS and PO$_4^{3-}$ and NO$_3^{-}$N in 2005 and 2006 suggest that these two constituents are transported into receiving water bodies attached to solids. The pH is likely to increase with increasing water temperatures, which were observed in 2004 (correlation coefficient 0.80) and in 2005 (correlation coefficient 0.45); but,
TABLE 7. Pearson correlation coefficients (statistical significance $p < 0.10$) between various water quality parameters*

(a) Results for 2004 (sample size 15)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TC</th>
<th>FC</th>
<th>EC</th>
<th>NH$_3$-N</th>
<th>TSS</th>
<th>Temp</th>
<th>pH</th>
<th>Cond</th>
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(b) Results for 2005 (sample size 18)

<table>
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<th>EC</th>
<th>NH$_3$-N</th>
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<th>BOD$_5$</th>
<th>TSS</th>
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<th>Temp</th>
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(c) Results for 2006 (sample size 14)

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*Temp: water temperature; Cond: conductivity; Turb: turbidity.
the same relationship could not be seen in 2006. It is generally acknowledged that FC decay increases with increasing pH (pH > 7). However, positive correlations between pH and FC (correlation coefficient 0.53) and E. coli (correlation coefficient 0.47) were found in the 2006 season, while no significant relationship between them was observed in the 2004 and 2005 season. The average pH values in these three seasons are 8.1, 8.6, and 9.6, respectively. The high pH value in 2006 may reflect high algal activity during this season. The high algal growth may protect microorganisms from natural environmental stresses such as sunlight. These results imply that the quality of a stormwater pond may also be affected by ecological characteristics such as algae and plant growth in stormwater ponds, which are also related to climatological variables.

Conclusions and Recommendations

The water quality assessment of stormwater reused for irrigating public lands was based on a three-year water quality monitoring program. Occasionally microbial levels after a heavy rain event as well as SAR levels exceeded applicable guidelines. However, the water quality observations indicated that the quality level of stormwater available for reuse generally meets the requirements for irrigation under dry periods and low flow conditions when watering is likely to be performed.

The correlations of stormwater quality and climatological variables were shown. It was found that climatic variables affect quality parameters such as TSS and nutrients in a more cumulative manner than others like microorganisms. The multiple linear regression models of FC and TSS represented the observations during the sampling seasons fairly well. Rainfall and wind were identified to have significant impacts on modelling the time series of FC, while temperature and wind played significant roles on TSS. The effect of rainfall on TSS might be masked by other climatological variables due to lack of TSS data for rain events in the 2004 season. Negative coefficients of wind in both FC and TSS regression models implied that wind-induced hydrodynamic disturbance did not influence surface water quality during the summer seasons.

Changes in precipitation/rainfall, temperature, and wind speed, etc, due to climate change are expected to influence water quality. A most recent study by Komatsu et al. (2007) assessed, using a modelling approach, the effect of climate change on lake water quality parameters, such as water temperature, DO, and nutrients, as well as aquatic ecosystems. Climatic variables projected by a Regional Climate Model were used as inputs in their study. In this study, the correlation and regression analyses provided insight into the way in which water quality can be a function of climatological variables. The identified relationships might be used to assess the impacts of future climate scenarios for the summer. Therefore, climate change should be taken into consideration to formulate a robust strategy for stormwater reuse over a long-term time frame. However, the impacts of wind on water quality and rainfall on TSS should be further verified.

It was noted that rain events significantly contributed to elevated microbiological levels in this pond. Although the irrigation system is prevented from watering for a couple of days after it is shut off due to rain events, it is necessary to technically evaluate the time period required to recommence irrigation in order to ensure public safety. The effect of rain events on microbiological quality should be further studied along with the removal efficiency of microorganisms of the pond. On the other hand, it is also necessary to investigate what is entering the pond under changing climatic conditions. In addition, better knowledge of fluid dynamics and ecological aspects would provide further understanding of the overall mechanism and removal efficiency of pollutants in stormwater ponds. Finally, climate change impacts on the ecological development and fluid dynamics, which are also associated with meteorological conditions, in stormwater ponds are other considerations to be investigated in a study of climate change impacts on water quality in these ponds.

Acknowledgments

The authors would like to thank the reviewers for their efforts and insightful comments which greatly improved this paper. This research was supported by the City of Calgary and the Canadian Climate Impacts and Adaptations Program (NRCAN). The authors would like to thank Francois J.-C. Bouchart, Liliana Bozic, Pamela Duncan, and Phillip Jerome of the City of Calgary for their contributions.

References


Received: 23 April 2007; accepted: 12 May 2008.
The Benefits of Biotreatment for Reducing the Effects of Pulp and Paper Mill Effluents on Fish Reproduction in Laboratory Tests

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Pulp and paper mill effluents have been reported to cause changes in reproductive indicators of fish in laboratory and field studies. These changes include reduced egg production and gonad size, and altered hormone levels and expression of secondary sex characteristics. We examined the performance of biotreatment plants for their potential in abating effects of pulp and paper mill effluents on fish reproduction under laboratory conditions. A bleached kraft mill effluent (BKME) treated in an aerated lagoon and a thermomechanical pulp mill effluent (TMPE) treated by aerobic sludge in a sequential batch reactor were selected for study. Mature fathead minnows (Pimephales promelas) were exposed to effluents before and after biotreatment under continuous renewal conditions for 21 days. Egg production was monitored daily, while morphometric parameters (length, weight, gonad size), secondary sexual characteristics, and steroid hormone and vitellogenin levels were measured at the end of the effluent exposure. The effluent from both mills before biotreatment impaired the reproductive capacity of minnows (egg production) at concentrations of 10 and 20% vol/vol, but not at 2% vol/vol. Exposure to biotreated effluents from both mills at concentrations of 2, 10, 20, and 40% vol/vol caused no significant differences in overall reproductive capacity of minnows as compared with controls. These results indicate that biotreatment can significantly improve the quality of a BKME and an effluent from a TMP mill with respect to the reproductive capacity of fish as determined in laboratory tests.

Key words: kraft, thermomechanical, effluent, treatment, reproduction, fishes

Introduction

There have been numerous reports that exposure of fish to pulp and paper mill effluents can cause changes in reproductive indicators such as secondary sexual characteristics, gonad size, sex hormone levels, and egg production (Hewitt et al. 2008). In Canada, the regulatory Environmental Effects Monitoring (EEM) program also showed that one consequence of fish exposure to mill effluents in the field may be smaller gonads that could affect overall reproductive capacity (Lowell et al. 2005). To address this issue, a multiphased research activity has been initiated with the ultimate goal of developing cost-effective mitigation options (Kovacs and Martel 2006). For this initiative, effluent quality is characterized by a standardized reproduction test with the adult fathead minnow (Pimephales promelas) (Ankley et al. 2001). The test includes several reproduction endpoints such as egg production and sex steroids. In the first phase, effluents from 10 mills were tested and it was found that effects (mainly vitellogenin induction in males) were not linked to a specific manufacturing process or type of effluent biotreatment (Martel et al. 2004; Kovacs et al. 2005). The second phase, currently ongoing, is aimed at the evaluation of remedial technologies.

The first activity regarding remedial technologies is to appraise the role of effluent biotreatment. There are three main reasons for this. First, biotreatment is essential to meet current regulatory limits on BOD and toxicity (Canadian Fisheries Act 1992). Second, previous studies have provided somewhat contradictory findings in that biotreatment was reported to result in improvement as well as worsening of effluent quality (Hewitt et al. 2008). Third, biotreatment holds the possibility of an industry-wide solution, irrespective of source(s)/cause(s) of effects, through optimization steps. The appraisal of the role of biotreatment is aimed at i) increasing our database as to how current biotreatment systems affect effluent quality and, using this information, ii) evaluating if the efficacy of the existing biotreatment systems can be improved to the point of eliminating effects on fish reproduction. This work focused on the evaluation of current biotreatment systems by comparing the effluents discharged by mills before and after biotreatment for their potential to affect fish reproduction.

Material and Methods

Mills Selected for Study

Two mills, one that produces newsprint by the thermomechanical pulp (TMP) process as well as deinked recycled pulp, and one that produces fine paper
from bleached kraft pulp, were selected for study. During the study period (November 2004), mean production at the newsprint mill was 842 tonnes per day. The pulp was made from 70% softwood chips (approximately 89% spruce, 10% fir, and 1% pine) and 30% de-inked recycled magazines. Wastewaters were screened to remove large debris, adjusted for pH, and supplemented with nutrients (phosphoric acid and urea) before biotreatment in a sequential batch reactor (SBR). Mean outflow from the SBR was 29,212 m$^3$ per day. The SBR consists of four basins functioning in sequence. During normal operation, the first basin is in filling mode, the second basin is in biological reaction mode, the third basin is in sedimentation mode, while the fourth basin is in discharge mode. The mean duration of a cycle is 5.5 hours. A fifth basin remains in standby for spill control in case of an emergency. During the biological reaction mode, diffusers inject fine bubbles of air in the reactor. Microorganisms use the oxygen and nutrients to degrade organic matter in the effluents. During sedimentation, aeration is interrupted and sludge is allowed to deposit to the bottom of the reactor leaving clarified effluent on the surface. The clarified effluent is discharged to a river, while part of the sludge is extracted from the reactor for incineration.

The bleached kraft mill in this study produced 1,600 tonnes/d of fine paper from a hardwood chip furnish composed of approximately 61% maple, 26% birch, and 13% aspen. Wood chips were pulped using a Kamyr modified continuous cooking digester followed by bleaching with a combination of 100% chlorine dioxide (D), caustic extraction (Eo), and peroxide (P) in a DEoD(P) sequence. Effluents from various sources in the mill were combined and screened to remove large debris before primary treatment in a clarifier, and biological treatment in an aerated lagoon with a hydraulic retention time of five days. Supplemental nutrients (phosphoric acid and urea) were added for biotreatment and the final effluent was discharged to a river at a mean rate of 65,000 m$^3$ per day.

**Effluent Sampling**

Effluent samples were collected between November 15th and 29th, 2004 and February 7th and 21st, 2005, respectively. Once a week for a three-week period, grab samples of combined mill effluent before (2,000 L) and after (3,000 L) biotreatment were taken by mill staff. The effluent samples were shipped in 1,000-L bulk containers lined with food-grade polyvinyl tubing cut in half longitudinally. The eggs were deposited by the female on the inside of the substrate where fertilization by the male took place. Spawning substrates were monitored for egg production daily. Hatching success and larvae survival were monitored from eggs produced by each group during one day of the week chosen randomly.

The test procedure was based on protocols described by Ankley et al. (2001). Before effluent exposures were started, mature minnows were distributed in 108 groups of two males and four females into aquariums containing six chambers and held for 21 to 25 days in well water. Each chamber contained two spawning substrates made from 8-cm lengths of 10-cm diameter food-grade polyvinyl tubing cut in half longitudinally. The eggs were placed in each aquarium (12.5 L), contained two males, four females, and two spawning substrates. A serial diluter system was used to deliver effluent concentration on a continuous basis and the volume replacement time was 2.4 hours in each aquarium. Throughout the experiment, every aquarium was aerated at a rate of 7.5 mL/min. The pH (7.5 to 8.4), temperature (25 ± 1°C), and dissolved oxygen (>65% saturation) were recorded daily. The accuracy of the concentrations delivered by the flow-
Effects of Biotreated Pulp Mill Effluents on Fish

through apparatus was verified daily by measuring the flows of water and effluent. The photoperiod throughout the study was 16 hours light and eight hours dark.

During the exposure phase of the experiment, egg production, fertility, and hatching success were monitored in the same way as during the pre-exposure period. At the end of the experiment, the secondary sexual characteristics were recorded and scored according to the criteria described earlier (Martel et al. 2004; Kovacs et al. 2005). Observations included colouration, banding, size of the dorsal pad, presence of a dorsal fin spot, number of nuptial tubercles, presence and development of an ovipositor, and body roundness. Some of these features are normally seen only in males or females, but each sex was examined for each feature. The minnows were then sacrificed, and measured for weight and length. These measurements were used to calculate condition factors (weight/length\(^3\)). The gonads were then removed and weighed. The gonad weights and body weights were used to calculate gonad somatic indices (GSIs; gonad weight/body weight x 100). The carcasses were homogenized in phosgel (0.04 M Na\(_2\)HPO\(_4\), 0.009 M NaH\(_2\)PO\(_4\), 0.1% gelatine, and 0.0002 M Thimerosal pH 7.6) buffer at 4°C and centrifuged at 3,100 x g for 10 minutes. The resulting supernatants were stored at -85°C for sex steroid and vitellogenin analysis.

Analysis for sex steroids was performed by enzyme immunoassay (EIA) after ether extraction of the supernatants, using the reagents and plates purchased from Cayman Chemical (Michigan, U.S.A.). The supernatants prepared from females were analyzed for estradiol and testosterone while the supernatants prepared from males were analyzed for testosterone only. The results of steroid analyses were expressed as picograms of steroid hormone per gram of fish. Vitellogenin analysis was conducted on male homogenates only using the fathead minnow vitellogenin enzyme immunoassay (EIA) kit from Biosense Laboratories (Bergen, Norway). The analysis is based on a sandwich EIA utilizing specific binding between antibodies and vitellogenin for quantification of minnow vitellogenin (VTG). Results of the vitellogenin analyses were expressed as nanograms VTG per gram of fish. All samples were assayed in duplicate.

Statistical Analyses

Statistical analyses were carried out with STATGRAPHICS Centurion XV Professional (StatPoint Inc., Herndon, Va.) and TOXSTAT version 3.5 (1996, Lincoln Research Associates, Bisbee, Ariz.) following the Environment Canada (2005) guidance document on statistical methods for toxicity tests. All statistical comparisons were made at the 5% significance level (\(p < 0.05\)). When necessary, the data were log transformed to meet assumptions of normality and homogeneity. When the data met assumptions of normality and homogeneity, the mean number of eggs produced per female per day, number of spawns, percent fertilization, and percent hatching from fertilized eggs were compared for significant differences by analysis of variance (ANOVA) with the aquarium being the experimental unit of replication. Differences in total body weight with length as a covariate, and gonad weight with body weight as a covariate were analysed by ANCOVA (analysis of covariance). Whole-body estradiol, testosterone, and VTG were compared for significant differences using an ANOVA model with aquarium as a nested factor. In cases when the ANOVA indicated a significant effluent-related effect, the Dunnett’s test was used to identify the specific effluent concentrations that were statistically significantly different from the control. When the data did not meet assumptions of normality and homogeneity, the nonparametric Kruskal-Wallis test, using the means of the particular endpoints from each replicate, was used to determine if the effluent exposure had a significant effect. When the Kruskal-Wallis test indicated significant effluent-related effects, the Steel’s Many-One Rank test was used to identify the specific effluent concentrations that caused a significant difference from the control.

Results

Chemical Analysis and Acute Lethal Toxicity of Effluents

Chemical analysis results for the kraft and TMP mill effluents are presented in Table 1. For the TMP mill effluent, biotreatment decreased the concentrations of BOD\(_5\), COD, and TSS by 96, 86, and 77%, respectively. Biotreatment reduced RFAs to nondetectable levels. For the kraft mill effluent, biotreatment reduced mean BOD\(_5\), COD, and TSS by 95, 58, and 41%, respectively. The kraft mill effluents were not analyzed for resin acids since only hardwood chips (not containing resin acids) were used for pulp production. None of the biotreated samples from the two mills caused any mortality of rainbow trout or Daphnia magna in 96 h and 48 h acute lethal tests. The TMP mill effluents before biotreatment had a mean 96h LC50 of 32% and 48h LC50 of 38% in tests with trout and Daphnia magna, respectively. The three effluents before biotreatment from the bleached kraft mill had a mean 96h LC50 of 22% in rainbow trout tests and 48h LC50 >40%, the highest concentration tested, in tests with Daphnia magna.

Adult Fathead Minnow Reproduction Test

Survival and morphometric indicators. No significant mortality was observed during both tests. The survival varied between 92 and 100% and was not dependent on effluent exposure. The condition factor of fish was unaffected (ANCOVA \(p > 0.05\)) by exposure to the effluents (data not shown). The average condition factor of the males and females across all treatments and controls were typical for the fathead minnow and ranged between 1.2 to 1.5 and 1.3 to 1.6, respectively.
TABLE 1. Chemical analysis and acute toxicity results for the effluents of this study before and after biotreatment

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*nd: not detected (detection limit 10 µg), na: not analyzed, RFA: resin and fatty acids, TSS: total suspended solids, BOD₅: biochemical oxygen demand over five days, COD: chemical oxygen demand, LC₅₀: lethal concentration to 50% of organisms.

In the case of the TMP mill study, the gonads of the males exposed to the final effluent before (ANCOVA \( p = 0.017; \) Dunnnett’s test) and after biotreatment (ANCOVA \( p < 0.004; \) Dunnnett’s test) were larger than controls at all concentrations except the 20% concentration of the effluent before biotreatment (Fig. 1). In the latter case, the gonad size of the males was smaller than in the control fish. The gonad size of the females was unaffected (ANCOVA \( p > 0.05 \)) by any of the TMP effluent concentrations tested (data not shown). In the case of the kraft mill study, the effluents did not affect gonad size in a statistically significant manner (ANCOVA \( p > 0.05 \)) for either sex. As was the case for condition factor, the GSIs of all the fish in this study were in the normal range. In the test with the TMP mill effluents, the mean GSI (detailed data not shown) of the control males was 1.1% and the mean GSIs of the effluent-exposed males was 1.15 to 2.0%. The mean GSI of the control females was 15.7% and the mean GSI of the effluent-exposed females ranged from 12.6 to 19.5%. In the test with the kraft mill effluents, the mean GSIs of the effluent-exposed males and females ranged from 1.2 to 1.6% and 12.7 to 17.3%, respectively. The mean GSI of the control males was 1.3% and the mean GSI of the control females was 16.5%.

**Egg production, fertilization, and hatching.** During the exposure phase of the experiment with TMP mill effluents, control replicates produced an average of 16 to 51 eggs/female/day (Table 2). Of these, the percentage of fertilized eggs varied between 51 and 87% while embryo survival varied between 36 and 62%. Exposure to 10 and 20% effluents before biotreatment caused a statistically significant decrease (ANOVA \( p < 0.0001; \) Dunnnett’s test) in egg production (Table 2), and this occurred instantaneously upon exposure to the effluent (see Fig. 2). This was linked to reduced spawning at the same concentrations (Kruskal-Wallis \( p = 0.005; \) Steel’s test). Despite the low egg production and spawning at these concentrations, fertilization and embryo survival were not found to differ from controls (Table 2). No effect on the number of spawning events, egg production, fertilization success, and embryo survival was found for the fish exposed to concentrations of 2, 10, 20, and 40% (vol/vol) biotreated effluent (Table 2).

During the effluent-exposure phase of the experiment with kraft mill effluents, control replicates produced an average of 9 to 15 eggs/female/day (Table 2) and, of these, the percentage of fertilized eggs varied between 26 and 75% while embryo survival varied between...
Effects of Biotreated Pulp Mill Effluents on Fish

The egg production by control fish, egg fertilization, and egg hatching were all lower than in the test with the TMP effluents as well as in our previous tests with biotreated effluents from 10 mills (Martel et al. 2004; Kovacs et al. 2005). As was the case with the TMP effluent, exposure to 10 and 20% concentrations of bleached kraft mill effluent (BKME) before biotreatment caused a statistically significant (Kruskal-Wallis $p = 0.02$; Steel's test) decrease in egg production (Table 2), and the effluent effect on egg production was evident from the first day of exposure to the effluent (see Fig. 3). Hatching success at 10% effluent before biotreatment was significantly better than the control (ANOVA $p = 0.007$, Dunnett’s test), but not at the other concentrations. The number of spawning events and percent of fertilized eggs were not affected by the effluent at concentrations of 2, 10, and 20% (Table 2). When compared with controls, exposure to the biotreated effluent had no significant effects on egg production, fertilization, or hatching at up to 40% concentration, the highest concentration tested (Table 2).

### Steroid hormones and vitellogenin

In the experiment conducted with the TMP mill effluents, the mean testosterone level in the male body homogenates of the controls was 1,430 pg/g (see Table 3). There were no

### TABLE 2. Egg production, number of spawning events, % fertilized eggs, and % hatching following exposure of fathead minnow to concentrations of combined mill effluents before and after biotreatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Eggs/female/day</th>
<th>Number of spawning events</th>
<th>Fertilized eggs, %</th>
<th>Hatched eggs, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TMP Effluent</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>33 (7.9)</td>
<td>14 (1.4)</td>
<td>67 (8.1)</td>
<td>49 (6.6)</td>
</tr>
<tr>
<td>After biotreatment, 2%</td>
<td>36 (5.5)</td>
<td>15 (1.5)</td>
<td>54 (5.1)</td>
<td></td>
</tr>
<tr>
<td>After biotreatment, 10%</td>
<td>24 (6.6)</td>
<td>13 (2)</td>
<td>49 (9.0)</td>
<td></td>
</tr>
<tr>
<td>After biotreatment, 20%</td>
<td>30 (5.6)</td>
<td>13 (1)</td>
<td>51 (8.2)</td>
<td></td>
</tr>
<tr>
<td>After biotreatment, 40%</td>
<td>24 (8)</td>
<td>12 (1.7)</td>
<td>46 (8.2)</td>
<td></td>
</tr>
<tr>
<td><strong>BKME</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12 (1.3)</td>
<td>8 (1.0)</td>
<td>52 (10)</td>
<td>28 (3.3)</td>
</tr>
<tr>
<td>After biotreatment, 2%</td>
<td>32 (11)</td>
<td>11 (1.4)</td>
<td>46 (16)</td>
<td>31 (9.1)</td>
</tr>
<tr>
<td>After biotreatment, 10%</td>
<td>31 (11)</td>
<td>13 (3)</td>
<td>39 (8.7)</td>
<td></td>
</tr>
<tr>
<td>After biotreatment, 20%</td>
<td>31 (10)</td>
<td>14 (2.8)</td>
<td>37 (7.8)</td>
<td></td>
</tr>
<tr>
<td>After biotreatment, 40%</td>
<td>23 (4.6)</td>
<td>12 (1.1)</td>
<td>29 (3.1)</td>
<td></td>
</tr>
</tbody>
</table>

*Results are expressed as means (standard error).
The numbers in brackets represent the range.
* Asterisk (*) indicates a statistically significant difference from the control ($p < 0.05$).
--- indicates that insufficient data was collected due to very low egg production.
significant differences in the males exposed to effluents before and after biotreatment. VTG levels in male body homogenates were significantly increased (Kruskall-Wallis \( p = 0.005 \); Steel’s test) at all concentrations for the test with effluent before biotreatment, and at 20 and 40% for the test with biotreated effluent (ANOVA \( p = 0.024 \); Dunnett’s test). Levels were increased 8- to 23-fold in fish exposed to effluents before biotreatment compared with controls (mean levels 145 ng/g). In the males exposed to biotreated effluent, at concentrations of 20 and 40%, the intensity of the response was lessened as VTG levels were only increased 5- to 6-fold over the controls. The females exposed to TMP effluents after biotreatment showed no significant differences in body homogenate levels of testosterone and estradiol in comparison with the controls (see Table 4). The levels of these hormones were, however, decreased in females exposed to the effluent before biotreatment at a concentration of 20% for testosterone (ANOVA \( p = 0.039 \); and Dunnett’s test), and at a 10 and 20% concentration for estradiol (ANOVA \( p < 0.001 \) and Dunnett’s test).

In fish exposed to kraft mill effluents, the mean testosterone level in the male body homogenates from the control group was 2,435 pg/g (see Table 3). The biotreated effluent and effluent before biotreatment caused no statistically significant differences. The vitellogenin levels in males exposed to 2, 10, and 20% effluent concentrations before biotreatment were about 4- to 30-times higher (Kruskall-Wallis \( p = 0.008 \); Steel’s test) than controls (mean 247 ng/g). Statistically significant (ANOVA \( p = 0.002 \); and Dunnett’s test) increases in vitellogenin levels were also measured in males exposed to 2, 10, 20, and

---

**TABLE 3.** Mean concentrations (standard error) of testosterone and vitellogenin in male fathead minnow exposed for 21 days to final mill effluents before and after biotreatment

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Before biotreatment</th>
<th>After biotreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2%</td>
<td>10%</td>
<td>20%</td>
</tr>
<tr>
<td><strong>TMP effluent</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone (^{b})</td>
<td>1430 (168)</td>
<td>709 (235)</td>
<td>948 (255)</td>
</tr>
<tr>
<td>Vitellogenin (^{c})</td>
<td>145 (45)</td>
<td>1134* (210)</td>
<td>3321* (499)</td>
</tr>
<tr>
<td><strong>BKME</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone (^{b})</td>
<td>2435 (392)</td>
<td>1348 (389)</td>
<td>954 (331)</td>
</tr>
<tr>
<td>Vitellogenin (^{c})</td>
<td>247 (33)</td>
<td>1093* (486)</td>
<td>7427* (1899)</td>
</tr>
</tbody>
</table>

\(^{a}\) Asterisk (*) indicates a statistically significant difference from the control (\( p < 0.05 \)).

\(^{b}\) Testosterone units: pg/g of body weight.

\(^{c}\) Vitellogenin units: ng/g of body weight.
Effects of Biotreated Pulp Mill Effluents on Fish

Primary sexual characteristics. When biotreated effluents were present before and after biotreatment, there were no indications of male primary sexual characteristics appearing in females, or of female primary sexual characteristics appearing in males. The TMP effluent before and after biotreatment had no effect on ovipositor length and body roundness of females, or dorsal fat pad, number of nuptial tubercles, and body colouration of males (data not shown). The kraft mill effluent before and after biotreatment also had no effect on the primary sexual characteristics of males. However, the kraft mill effluent before biotreatment caused a statistically significant reduction (Kruskal-Wallis $p = 0.012$; Steel’s test) in the length of the ovipositor in females exposed to 10 and 20% effluent concentrations (length of controls, 1.9 mm; length of females exposed to 10 and 20% effluent concentrations were 1.3 and 1.1 mm, respectively). No other differences were noted in females exposed to the Kraft mill effluents.

Discussion

The Effect of Biotreatment

The main aim of this study was to determine how the effluent biotreatment systems installed at existing mills influence effluent quality as it relates to fish reproduction. For this purpose, effluents were sampled before and after biotreatment at two mills, and these effluents were tested in the laboratory where various reproductive indicators of effluent-exposed fathead minnow were compared with control fish. The results showed that biotreatment eliminated/reduced effluent-related effects in the laboratory tests as can be seen in the summary of results shown in Tables 5 and 6. For the TMP mill effluent and the BKME, of the 12 endpoints listed in the tables, six and four, respectively, were deleteriously affected by ≥2% concentrations of the effluents before biotreatment (increased gonad size was not considered to be a deleterious effect). After biotreatment, both effluents affected only one endpoint (VTG induction in males) in a deleterious manner. The other endpoints were unaffected by up to 40% concentration of biotreated effluents. Exposure to biotreated effluents did not trigger any new responses in the fish that were not already seen in fish exposed to effluents before biotreatment. As well, none of the endpoints worsened as a result of exposure to biotreated effluents in comparison with exposure to effluents before biotreatment.

The greatest benefit of biotreatment was in reducing effects on egg production, the key indicator of overall reproductive capacity. Prior to biotreatment, the TMP effluent and the BKME caused a virtual shutdown of egg production at 10 and 20% concentrations, whereas the biotreated effluents from the two mills did not affect egg production at up to 40% concentration. The control egg production in the test with the effluents from the bleached kraft mill was lower than in the test with the TMP effluent or in our previous tests with biotreated effluents from 10 mills (Martel et al. 2004; Kovacs et al..

40% concentrations of biotreated effluents, but these were of generally lower intensities resulting in respective increases of 12-, 8-, 6-, and 10-times over mean control levels. In females, testosterone and estradiol levels were unaffected by effluent exposure (see Table 4).

Secondary sexual characteristics. In tests with both effluents before and after biotreatment, there were no indications of male secondary sexual characteristics appearing in females, or of female secondary sexual characteristics appearing in males. The TMP effluent before and after biotreatment had no effect on ovipositor length and body roundness of females, or dorsal fat pad, number of nuptial tubercles, and body colouration of males (data not shown). The Kraft mill effluent before and after biotreatment also had no effect on the secondary sexual characteristics of males. However, the Kraft mill effluent before biotreatment caused a statistically significant reduction (Kruskal-Wallis $p = 0.012$; Steel’s test) in the length of the ovipositor in females exposed to 10 and 20% effluent concentrations (length of controls, 1.9 mm; length of females exposed to 10 and 20% effluent concentrations were 1.3 and 1.1 mm, respectively). No other differences were noted in females exposed to the Kraft mill effluents.

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### Table 4. Mean concentrations (standard error) of testosterone and estradiol in female fathead minnow exposed for 21 days to final mill effluents before and after biotreatment.

<table>
<thead>
<tr>
<th>Effluent</th>
<th>Control</th>
<th>Before biotreatment</th>
<th>After biotreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2%</td>
<td>10%</td>
</tr>
<tr>
<td><strong>TMP effluent</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td>1115 (274)</td>
<td>849</td>
<td>464</td>
</tr>
<tr>
<td>Estradiol</td>
<td>2910 (565)</td>
<td>1677</td>
<td>1169*</td>
</tr>
<tr>
<td><strong>BKME</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td>2219 (589)</td>
<td>1731</td>
<td>1478</td>
</tr>
<tr>
<td>Estradiol</td>
<td>3411 (451)</td>
<td>3404</td>
<td>2676</td>
</tr>
</tbody>
</table>

* Asterisk (*) indicates a statistically significant difference from the control ($p < 0.05$).

b Testosterone units: pg/g of body weight.

c Estradiol units: ng/g of body weight.
We don't know the reason for this and can only speculate that it may have been due to the fact that the fish were 16 months old, whereas in the other tests the fish were around 12 months old.

Based on the results of this study, it was difficult to make a sound assessment of potential effluent-related effects on egg fertilization and hatching. While effluent-exposure caused no statistically significant differences, and this is consistent with our previous studies with numerous mill effluents (Martel et al. 2004; Kovacs et al. 2005), both endpoints were atypically low in control as well as effluent-exposed groups. We do not have an explanation for the poor fertilization and hatching other than the observation of some contamination of the eggs with fungus and possibly filamentous bacteria. The eggs produced by the fish in the experiments were placed in well water and were kept in the same room as the experimental aquaria. It is possible that microorganisms from the effluents contaminated the eggs. We did observe some growth of filamentous bacteria on the sides of the tanks containing the TMP and bleached kraft mill effluents prior to biotreatment. Despite the abnormal fertilization and hatching results, we consider that the rest of the results reported in this manuscript are nonetheless representative of the potential effects of pulp mill effluents. This is based on the observation that, upon dissection of the fish at the end of the exposures, we found no evidence of external or internal pathologies and

<table>
<thead>
<tr>
<th>Reproductive endpoint</th>
<th>Effluent before biotreatment</th>
<th>Biotreated effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg production</td>
<td>– (±10%)</td>
<td>0</td>
</tr>
<tr>
<td>Spawning events</td>
<td>– (±10%)</td>
<td>0</td>
</tr>
<tr>
<td>Gonad weight, males</td>
<td>+ (2% and 10%)</td>
<td>+ (±2%)</td>
</tr>
<tr>
<td>Gonad weight, females</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Testosterone, males</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Testosterone, females</td>
<td>– (±20%)</td>
<td>0</td>
</tr>
<tr>
<td>Vitellogenin males</td>
<td>+ (±2%)</td>
<td>+ (±20%)</td>
</tr>
<tr>
<td>Estradiol, females</td>
<td>– (±10%)</td>
<td>0</td>
</tr>
<tr>
<td>2* sexual characteristics, males</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2* sexual characteristics, females</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*“-” = statistically significant (p < 0.05) decrease; “+” = statistically significant (p < 0.05) increase; “0” = not affected by effluent exposure.

**TABLE 6.** Summary of the reproductive endpoints for the fathead minnow exposed in the laboratory to BKME before and after biotreatment.

<table>
<thead>
<tr>
<th>Reproductive endpoint</th>
<th>Effluent before biotreatment</th>
<th>Biotreated Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg production</td>
<td>– (±10%)</td>
<td>0</td>
</tr>
<tr>
<td>Spawning events</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gonad weight, males</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gonad weight, females</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Testosterone, males</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Testosterone, females</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vitellogenin males</td>
<td>+ (±2%)</td>
<td>+ (±2%)</td>
</tr>
<tr>
<td>Estradiol, females</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2* sexual characteristics, males</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2* sexual characteristics, females</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*“-” = statistically significant (p < 0.05) decrease; “+” = statistically significant (p < 0.05) increase; “0” = not affected by effluent exposure.

**TABLE 5.** Summary of reproductive endpoints for the fathead minnow exposed in the laboratory to TMP newsprint mill effluent before and after biotreatment.

*Effluent concentration indicated in parentheses.

**2* = secondary.**
other morphological and biochemical parameters were within normal range.

In addition to the beneficial effect on egg production, biotreatment was found to be effective in eliminating i) TMP mill effluent-related reductions of steroid hormones in females as well as a reduction of gonad size in males, and ii) BKME-related effects on egg hatching as well as ovipositor length in females. Finally, biotreatment reduced the degree of VTG inductions in males exposed to both the TMP effluent and the BKME.

The induction of VTG in males was the only remaining (deleterious) effect of the effluents from the two mills after biotreatment. When using the same test for surveying biotreated effluents from 10 mills, the VTG response was found to be the most common consequence of effluent-exposure, occurring in about 50% of the cases (Martel et al. 2004; Kovacs et al. 2005). Clearly, the effluents continue to contain substances that can cause this effect even after biotreatment, albeit to a lesser degree than exists in effluents before biotreatment. The VTG response in the tests with biotreated effluents (up to 40% concentration) did not influence the overall egg production.

To date, only limited work has been done that directly compares the potential of a pulp mill effluent to cause reproductive effects in fish before and after biotreatment. And the limited work provided somewhat contradictory findings as to the specific role of biotreatment regarding effluent effects on fish reproduction (as reviewed by Hewitt et al. 2008). In one case, for example, biotreatment was reported to increase the effect of a BKME on plasma testosterone levels in fish (Dubé et al. 2002), while in other cases biotreatment was found to have beneficial influences as occurred for the effects of a i) TMP mill effluent on plasma testosterone levels in fish (Johnsen et al. 2000), and ii) a kraft/TMP mill effluent in causing changes in secondary sexual characteristics (Ellis et al. 2003). Because of these contradictory findings, the actual role of biotreatment as a possible mitigation option was questionable. Part of the reason for the contradictory findings of the previous studies may have been the consequence of work with different species and effluents, testing strategies (e.g., single concentration tests), and endpoints measured. The findings of this study on the other hand, where multiple concentrations of effluents were tested before and after biotreatment by the same test, provided more clear evidence regarding the potential benefit of biotreatment.

Industry Significance

For an industry that is trying to reduce or eliminate specific effects associated with its discharges, information about what operational changes could achieve this goal is vital. Hence, another aim of this study was to search for leads, especially with respect to biotreatment, that could be used by industry to further improve effluent quality in terms of effects on fish reproduction. Two key factors that can influence effluent quality are the manufacturing process, including the wood furnish that has long been suspected to be the source of substances affecting fish reproduction (Hewitt et al. 2008) and the type of biotreatment system for treating wastewaters. The two mills selected for this study represent the major types of pulping processes and biotreatment systems in Canada. The two major manufacturing processes are mechanical pulping (e.g., TMP) for producing newsprint, and chemical pulping (e.g., bleached kraft) for making pulp that can be used for fine paper production. The two broad categories of biotreatment systems installed by mills in Canada include aerated lagoons and activated sludge systems (Paice et al. 2003; Mahmood and Paice 2006). The latter encompass mostly conventional activated sludge plants as well as less frequent pure oxygen-based activated sludge systems and sequential batch reactors. An important starting point for the search in eliminating/reducing effluent-related effects on fish reproduction is to gain insights into the role of the manufacturing processes and type of biotreatment systems currently in use.

The parameters given in Table 1 provide the conventional descriptors of effluent quality. Despite differences in wood furnish, water usage, and pulping process, the concentration of BOD and TSS was largely similar in the discharges from the two mills prior to biotreatment. The COD was about 1.3 times greater in the effluent from the bleached kraft mill and, typically, the COD in kraft mill effluents is greater than in TMP mill effluents. The acute lethal toxicity of the effluents from the two mills before biotreatment was comparable in tests with rainbow trout, and the BKME was less toxic in tests with Daphnia magna. Biotreatment of the two effluents resulted in similar concentrations of BOD (~95% reduction) and elimination of acute lethal toxicity to rainbow trout and Daphnia magna. After biotreatment, the BKME had higher concentrations of COD (~3.8x) and TSS (~2.5x) than the TMP mill effluent, and this may have been the result of differences in the treatment systems (likely the case for TSS as activated sludge systems in general select for better settling biosolids) or in the nature of the material in the effluent before biotreatment. The COD in the effluent from the bleached kraft mill most likely contained more recalcitrant material, such as lignin and chloro-lignin, and this may have contributed to differences in the COD reduction at the kraft (~60%) and TMP (~85%) mills. Clearly, both the manufacturing processes and the type of biotreatment systems have an influence on the levels of some conventional parameters in the final discharge from mills.

The conventional environmental parameters in Table 1 that are typically used to characterize effluent quality and biotreatment efficiencies, however, do not always reflect well the effluent quality in terms of effects on fish reproduction. Previous work using the same fathead minnow test as in this study has shown that the effects of biotreated mill effluents on various reproductive indicators in fish are not dependent on
the manufacturing process or the type of biotreatment system (Martel et al. 2004; Kovacs et al. 2005). This was essentially confirmed in this study. After biotreatment, there was no difference in the effects of the effluents from the two mills, even though the types of biotreatment systems were substantially different. However, in terms of the manufacturing process, the TMP mill effluent before biotreatment in this study affected more of the reproductive indicators in fish than the effluent before biotreatment from the kraft mill (see Tables 4 and 5). The greater effects were possibly the consequence of the lower water usage at the TMP mill (~35 m$^3$/adt versus ~40 m$^3$/adt for the kraft mill) that resulted in greater concentration of contaminants, or it may have been due to the different wood furnish, softwood in the case of the TMP mill and hardwood in the case of the kraft mill.

Clearly, the higher COD concentration in the BKME, representing recalcitrant material, as well as the higher TSS were not critical factors in affecting egg production, and this suggests that substances affecting egg production and other reproductive indicators are most likely soluble and relatively small molecular weight in nature. There is some evidence for this from the findings of this and earlier studies. Prior to biotreatment, the effluents from the two mills in this study affected egg production at 10 and 20% concentrations immediately upon the exposure of the fish to these solutions (see Fig. 2 and 3 for cumulative egg production). The sudden cessation of egg production was also observed in our previous tests with effluents both before and after biotreatment (Martel et al. 2004; Kovacs et al. 2007), and this observation indicates a very rapid (acute) effect that is suggestive of direct uptake from the water as opposed to the effect of material (e.g., solids or sediments) that requires bioaccumulation. Previous work by others in the laboratory as well as in the field have also provided findings indicating that mill effluent-related effects on reproductive indictors in fish are most likely the consequence of direct uptake from the water (Hewitt et al. 2008).

Overall, the results of this work show that two major types of biotreatment systems currently in use (aerated lagoon and activated sludge/sequential batch reactor) can have beneficial effects not only in meeting regulatory limits (BOD, TSS, and acute lethal toxicity) but also in mitigating effluent-related effects on fish reproduction. Despite this, some effluent-related effects such as the VTG response in this study and effects on egg production in laboratory studies at 100% effluent concentration (Kovacs et al. 2007) still occur. More work is required to ascertain that biological treatment can be efficient in reducing reproductive effects over the range of treatment options and manufacturing processes that exist throughout the industry. As well, smaller gonads in wild fish living in effluent-exposed zones continue to be reported by EEM studies (Lowell et al. 2005). Collectively, this indicates that still additional improvements in effluent quality may be needed. The beneficial effect by the typical biotreatment systems seen in this study point out that it may not be necessary to change the type of biotreatment systems currently in use, but, rather, to explore further their mitigating potential, irrespective of what the causative agent(s) for changes in the reproductive indicators of fish may be. Consequently, additional work may now be undertaken to further characterize the performance of biotreatment systems and progress toward their optimization to determine if all effluent-related reproductive effects can be eliminated or whether in-plant measures will also be required.

Conclusions

- Biotreatment in a sequential batch reactor and an aerated lagoon was found to improve the quality of the effluent from a TMP and a bleached kraft mill, respectively, in terms of overall reproductive capacity (egg production) of the fathead minnow in laboratory tests.
- The beneficial effect of biotreatment was independent of both the manufacturing process and the type of effluent biotreatment used by the two mills.
- While biotreatment eliminated effluent-related effects on overall reproductive capacity, vitellogenin expression in males was diminished but remained significant even after effluent biotreatment. This indicates that mill effluents may have multiple causes of effects, and additional work will be needed to assess if biotreatment can be further optimized to eliminate all effluent-related effects on fish throughout the industry.

Acknowledgments

The authors wish to acknowledge the technical support of Tatyana Yurchuck, Maria Ricci, Sharon Gibbons, and Robert Traversari. Robert Poole of DataPoole (Pointe-Claire, Quebec) provided valuable assistance with statistical analysis and Mike Paice of FPInnovations-Paprican made useful suggestions for improving the manuscript. This work was funded in part by the members of the Quebec Forest Industry Council (QFIC) and Member Companies of FPInnovations - Paprican.

References


Received: 28 September 2007; accepted: 14 May 2008.
Quantification of Plant Sterols in Pulp and Paper Mill Effluents

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Pulp and paper mill effluents (PPMEs) may contain high levels of otherwise naturally occurring organic pollutants such as plant sterols, which are suspected endocrine disrupting chemicals. Exposure to such chemicals may cause various physiological and morphological abnormalities that have been reported in the fish and other aquatic life inhabiting PPME receiving waters. Plant sterols, or phytosterols, form a constituent of wood extractives that may be released into the effluents during the pulping and paper making processes. Isolation and analysis of sterols from the complex mixture of PPMEs is challenging and standard analytical protocols do not exist. The need for having a reliable method for analyzing a particular environmental contaminant such as plant sterols cannot be overemphasized. In the present study a technique was modified for reliable analysis of PPME sterols. The technique involves liquid-liquid extractions using methyl-t-butyl ether and trimethyl-silylation derivatizations of the extracted sterols. Identification and quantification of the PPME sterols were accomplished by gas chromatography and mass spectrometry. Analytical problems were resolved by conducting multiple extractions, drying the sterol extracts, and redissolving and silylating the extracts at an increased derivatization temperature of 70°C. This shortened the suggested incubation period from 12 to 4 h. The modified technique offered improved method sensitivity and reproducibility, and successfully quantified campesterol, β-sitosterol, β-sitostanol, stigmasterol, stigmastanol, cholesterol, and ergosterol in PPMEs. Primary and secondary treated PPME analyses suggested 800 ± 190 μg/L total sterols in primary effluents, and 211 ± 90 μg/L in biologically treated final effluents. β-Sitosterol, β-sitostanol, and campesterol alone accounted for about 80% of the total sterols. A general comparison of the sterols in primary and secondary effluents suggested about 73% removal across the secondary treatment systems sampled.

Key words: pulp and paper mill effluents, endocrine disrupting chemicals, plant sterols/phytosterols analysis, silylation, β-sitosterol, industrial wastewater treatment

Introduction

Sterols occur in both softwood and hardwood species (Holl and Goller 1982; Gao et al. 1995), usually with β-sitosterol as a major component, and have been detected in receiving waters downstream of pulp and paper mills (Cook et al. 1997; Mahmood-Khan and Hall 2003). Plant sterols are suspected endocrine disrupting chemicals or hormonally active agents that may be estrogenic or sometimes androgenic to fish (Van Der Kraak and MacLatchy 1994; Zacharewski et al. 1995; Lehtinen et al. 1999). β-Sitosterol and abietic acid present in pulp mill effluents (Mellanen et al. 1999; Oikari and Holmbom 1996) may be directly or indirectly responsible for estrogenicity in debarking effluents (Mellanen et al. 1996, 1999) as assessed by vitellogenin expression bioassays. Stromberg et al. (1996) also noted the presence of such phytosterols in acetone extracts of elemental chlorine free and totally chlorine free oxygen bleached modern pulp mill effluents. β-Sitosterol, associated with bleached kraft mill effluents, and coprostanol significantly induced vitellogenin in rainbow trout at exposure levels as low as 75 μg/L (Fernandez et al. 2007). Additionally, these sterols have been observed to reduce pregnenolone and cholesterol levels in immature trout (Tremblay and Van Der Kraak 1999). Therefore, it is important to examine the presence of phytosterols in pulp and paper mill effluents (PPMEs) as well as in the receiving waters.

Quantitative analysis of plant sterols from PPMEs is complex. PPMEs contain a mixture of dissolved wood extractives, methanol, acetone, organic acids, cellulose and lignin products, sulphides and mercaptains, adsorbable organic halides, sterols, steryl esters, triglycerides, and other lipophilic compounds (Mimms et al. 1989; Orsa and Holmbom 1994). Standard analytical protocols have not been published for isolating and analyzing sterols. However, several analytical techniques have been proposed for wood extractives in PPMEs (Holmbom 1980; Ekman and Holmbom 1989; Alvarado et al. 1992), and from wood pulps (Sithole 1993; Gao et al. 1995; Peng et al. 1999). These procedures generally focused on lipophilic fractions collectively and analyzed sterols indirectly. None was developed exclusively for sterols and most of the procedures appeared laborious and expensive for analyzing large numbers of effluent samples in a reasonable amount of time. Hence, a simple and reliable analytical technique is required for sterols in PPMEs and other water environments.
Reported literature indicates that sterol isolation attempts using adsorption/desorption, steam distillation, solid-phase extraction (SPE), and liquid-liquid extractions (Method 85.02 National Council for Air and Stream Improvement, NCASI 1986) did not work well (NCASI 1997). Another SPE method separated sterols just qualitatively (Chen et al. 1994). Mosby et al. 2000 suggested an SPE technique that was suitable for white water matrices. McKague and Reeve 2003 used an accelerated solvent extraction with toluene refluxing at 100°C for samples containing solids. However, solvent extractions and gas chromatography have been recommended for sterols (Holmbom 1980; Ekman and Holmbom 1989; Suckling et al. 1990; Sithole 1993; Cocito and Delfini 1994; Gao et al. 1995; Elhmmali et al. 2000). Orsa and Holmbom (1994) preferred MTBE (methyl-t-butyl ether) over other solvents for higher yields of lipophilic extractives. Magnus et al. (2000), Cook et al. (1997), and NCASI (1997) also found MTBE extractions relatively effective for isolating PPME sterols with subsequent gas chromatography and mass spectrometry (GC/MS). This method was selected by Mahmood-Khan and Hall (2003) in a previous study; however, certain analytical problems were encountered that limited the usefulness of the technique. Inefficient silylation probably caused analytical problems and quantification errors due to the poor chromatography of the underivatized sterols. Therefore, a considerable effort was invested to develop a simple and reliable technique for quantifying sterols in PPMEs. The present study focuses particularly on the analytical problems and the modifications that were necessary for the successful application of the technique.

Due, in part, to the analytical challenges involved, specific data regarding the sterol concentrations before and after treatments are not readily available. Considerable differences exist in the reported levels of PPME sterols; Cook et al. (1997) reported from zero to 90% removal for nine mills using biological secondary effluent treatment systems. Sterol discharge estimates varied from 7 to 29 g/adt (grams per air dried ton of pulp) in treated effluents. Magnus et al. (2000) reported about 50% biodegradation of sterols across a high-efficiency compact reactor treating integrated mill effluents. Secondary treatment at an elemental chlorine free bleached kraft mill achieved high removal of extractives, but the estimated biodegradation/transformation of sterols was only 41% (Kostamo and Kukkonen 2003). A typical pulp mill producing 1,000 adt/day may discharge as much as 29 kg of sterols per day (Mahmood-Khan and Hall 2003).

The technique modified and developed in the present study was applied to analyze sterols in primary and secondary treated effluents from UNOX-AST (Union Carbide pure oxygen-activated sludge treatment) systems at two Canadian pulp mills. However, the main objective of the present study was to develop a simple and robust technique for analyzing sterols in PPMEs. Further studies related to this project shall focus on the effectiveness of specific secondary treatment systems for sterol removal, generate information important for the design and operation of treatment facilities, and estimate effluent sterols associated with solids as well as the environmental discharge of such pollutants.

Materials and Methods

Analyte Selection, pH, and Extraction Mode

Published literature (Ekman and Holmbom 1989; Suckling et al. 1990; Cocito and Delfini 1994; Cook et al. 1997; Magnus et al. 2000) and a survey of two Canadian mills (Mahmood-Khan and Hall 2003) suggested possible presence of ß-sitosterol, ß-sitostanol, stigmastanol, stigmasterol, ergosterol, and campesterol in the selected mill effluents. Authentic sterol standards were obtained from Sigma-Aldrich Ltd., Oakville, Ontario, Canada.

Near neutral pH (6 to 7) whole mill effluents were selected for sterol extractions to minimize the potential interferences due to extraneous organics in PPMEs (Orsa and Holmbom 1994; NCASI 1997). Selected samples of filtered solids and filtrate were also analyzed for quality control. However, liquid/liquid extractions of the whole effluent gave comparable or improved results to those obtained through Soxhlet extraction of filtered solids in combination with liquid/liquid extraction of the filtrate (data not shown).

Liquid/Liquid Extraction

For effluent samples, a 25 or 100 mL aliquot (25 mL for primary and 100 mL for secondary treated effluents) was collected and extracted with 15 or 40 mL of MTBE (15 mL for primary and 40 mL for secondary treated effluents). The extractions were completed in prefried clean Pyrex glass centrifuge tubes fitted with Teflon-lined screw caps. The PPME and MTBE mixture was shaken vigorously by hand and then by using a mechanical shaker (Innova Model # 4230, New Brunswick Scientific, Edison, N.J., U.S.A.) simulating wrist action shaking for 15 to 20 minutes. The contents were allowed to settle for 10 minutes and then centrifuged for 10 minutes at a relative centrifugal force of about 1,600 x g. The supernatant was collected and the procedure was repeated two times to recover most (~95%) of the sterols.

For sludge samples, 2 to 5 mL (2 mL for thick and 5 mL for dilute sludge or mixed liquor) were extracted three times using a combination of 4 mL of MTBE and 1 mL of methanol by the wrist-action mechanical shaker for 15 to 20 minutes before centrifuging for 15 to 20 minutes at about 1,600 x g. Alternatively, freeze-drying and 12 h Soxhlet extractions with MTBE were attempted without improved results.

The solvent extracts from each sample were collected. Water was removed by adding anhydrous magnesium sulfate which was then removed by filtering with two rinses of MTBE. The extracts were concentrated to about

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0.5 mL by rotary evaporation, transferred to 5-mL glass reaction vials, and reduced to dryness using nitrogen.

**Silylation Derivatization**

The dried extracts were reconstituted in 100 μL of hexane and about 50 μL of MTBE and suspended, to which was added 10 to 15 μL of BSTFA (N, O-Bis trimethylsilyl-trifluoroacetamide) per μg of the estimated sterol content based upon preliminary survey results. These were thoroughly mixed by vortex and incubated at temperatures and times as described below.

**Internal Standard and Surrogate**

Dotriacontane, not a constituent of PPMEs, was used as an internal standard (Cook et al. 1997). Dotriacontane does not interfere with sterol peaks and can account for GC performance and response. Its molecular weight (450 g/mole) is similar to those of the silylated phytosterols (466 to 482 g/mole). Cholesterol was selected as a recovery standard or surrogate to monitor the extraction efficiencies and other losses to the glassware during the sample extraction and preparation. A number of chemicals that were initially thought to be suitable surrogates were employed, but they did not prove successful. The recovery standard was added to PPME samples before the liquid-liquid extractions.

After silylation, the sterol extracts were dried again using nitrogen. The extracts were then redissolved in (water free) MTBE containing the internal standard. Final extract volumes were made up to 1 mL each in clean GC vials that were sealed by clamping using Teflon lined caps and loaded on to the GC auto-sampler.

**Gas Chromatography/Mass Spectrometry (GC/MS) Analysis**

The silylated sterol extracts were quantified using an HP-6890 Gas Chromatograph/HP-5973 Mass Spectrometer (GC/MS) with a 29-m J&W DB-5 capillary column with 0.25-μm chemical coating and 0.25-mm internal diameter. The GC oven program was 130°C, 1 min hold; (Ramp 1) to 285°C at 15°C/min, 3 min hold; (Ramp 2) to 310°C at 2°C/min, 1 min hold; and post run, 315°C, 3 min hold; 1 μL was injected with an auto-sampler, in a splitless fashion using a 10-μL syringe, the inlet temperature was 290°C, the carrier gas was helium at 11.2 psi, and the mass selective detector was at 280°C. The GC conditions were optimized for maximum sensitivity and minimum peak overlap, and selected for sharper and higher peaks, improved sensitivity, and reduced peak overlapping.

**PPME Sampling**

Sterol working standards in MTBE were used during the development of the analytical procedure. After the analytical problems were mostly resolved, primary and final treated PPMEs were collected on a weekly basis from two Canadian pulp and paper mills. The mills used UNOX-AST (Union Carbide pure oxygen-activated sludge treatment) bioreactors for their secondary wastewater treatment. The PPME samples were shipped in Nalgene plastic containers and stored at 4°C for 1 or 2 days before analysis.

During the sampling period, Mill A produced kraft and newsprint pulp, using 100% hemlock and a mix of spruce, pine, and fir respectively, with a bleaching sequence of ODeopDED (oxygen delignification, chlorine dioxide, extraction with oxygen and peroxide, chlorine dioxide, extraction, chlorine dioxide). Mill B produced kraft pulp with a wood furnish of about 70% hemlock, 15% cedar, 15% fir and followed a bleaching sequence of DEopD (chlorine dioxide, extraction with oxygen and peroxide, chlorine dioxide).

**Results and Discussion**

Analytical problems were encountered with the selected method, and which resulted in the development of a modified technique for analyzing sterols in PPMEs.

**Silylation of Extracted Sterols**

Silylation derivatization is expected to improve the chromatographic resolution and peak symmetry by substituting a trimethylsilyl group (Si(CH₃)₃) for an active H, which reduces the polarity and hydrogen bonding potential, as well as increases stability and volatility of the derivatives. Initially, the extracted sterols were derivatized at room temperature with overnight incubation as recommended (Cook et al. 1997). The method produced poor quality standard curves and produced qualitative results. Identification of underivatized sterol peaks in the chromatograms indicated derivatization problems. Therefore, the derivatization temperature was increased from room temperature to 40°C, and the incubation time was kept at 12 h or overnight. Mass spectrometry (results not shown here) confirmed that the derivatization of the extracted sterols was not attained effectively by overnight leaving of the mixture of sterol extracts in contact with BSTFA, either at room temperature or at an elevated temperature of 40°C.

Researchers have different recommendations for silylation, i.e., overnight incubation at room temperature (Cook et al. 1997), 20 min at 70°C (Orsa and Holmbom 1994), and 1 h at room temperature (NCASI 1997). Therefore, a number of silylation conditions were tested in this study, including overnight incubations at room temperature, and incubations for 1 to 24 h at 40, 65, and 70°C. The results are shown in Fig. 1 to 5. Standard curves could not be constructed for incubations conducted at 40°C. Therefore, results have not been shown for this temperature.

When the reaction temperature was increased from 40 to 65°C, most of the underivatized sterol peaks
Mahmood-Khan and Hall

disappeared from the chromatograms, especially at lower sterol concentrations (10 to 50 μg/L). However, at higher concentrations (70 to 250 μg/L), small underivatized sterol peaks could still be detected at the 65°C incubation. Hence, at 65°C, complete silylation was not guaranteed after 1 or 3 h (Fig. 1). The underivatized sterols may tend to stick in the GC column, reducing the analytical recoveries, and this was probably why the underivatized peaks were absent at low concentrations. The reaction was then conducted at 70°C for 1 and 3 h. No underivatized peaks were detected at any concentration tested at 70°C, but the silylated sterol peak areas increased for β-sitosterol and cholesterol with an increase in reaction time (Fig. 2), demonstrating that complete derivatization of the extracted sterols was not accomplished in 1 h.

Subsequently, the reaction was proceeded for up to 24 h, and samples were taken from the same batch reactor at different times and analyzed for silylated sterols (Fig. 3). The results show that silylation approached completion after about 3 h, causing the peak areas to reach their maximum values that declined to relatively steady values after 5 h of incubation (Fig. 3). For a given temperature, different sterols followed a similar trend with incubation time. Therefore, two sterols were used to indicate the trends of other sterols. A moderately reversible nature of the reaction was also indicated by some decline in peak areas of silylated cholesterol and β-sitosterol after 5 h at 70°C (Fig. 4). When the silylation reaction was allowed to proceed for 24 h, the normalized peak areas of silylated plant sterols did not improve significantly (Fig. 5). The prolonged incubation at 70°C did not prove to be detrimental for silylation, as the normalized peak area of silylated sterols slightly increased after 24 h. It was further confirmed that all the tested sterols followed similar trends (Fig. 5).

The peak areas of silylated cholesterol and stigmasterol approached near maximum values at about 3.5 to 4 h of incubation at 70°C (Fig. 4). Hence, silylation conditions of 3.5 to 4 h of incubation at 70°C were selected for optimum silylation. The modified silylation conditions reduced the recommended incubation time of 12 h to 3.5 to 4 h for each batch, and increased the peak areas of extracted sterols. In further experiments, it was observed that the sterol standards were derivatized effectively in 3 to 3.5 h, but for some PPME and sludge samples, underivatized sterol peaks were identified when the extracts were incubated for less than 3.5 h. These problems were addressed by increasing the incubation time to 4 h at the same temperature (70°C), making the sterol extracts free of water and keeping the extracted sterols dissolved in a mixed solvent composed of the silylation agent, hexane, and a small amount (50 to 100 μL) of MTBE.

**Calibration and Quantification**

The analysis revealed that each sterol required its own calibration curve for quantification (Fig. 5). As stated

**Fig. 1.** Peak area and silylation time for β-sitosterol and cholesterol derivatives at 65°C.

**Fig. 2.** Peak area and silylation time for β-sitosterol and cholesterol derivatives at 70°C.

**Fig. 3.** Peak area of cholesterol (Chole) and β-sitosterol (β-Sito) (silylation at 70°C). -I = trial-I; -II = trial II; Ave = mean value.
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Fig. 4. Peak area of cholesterol (Chole) and stigmasterol (Stigma) (silylation at 70°C). -I = trial I; -II = trial II; Ave = mean value.

Fig. 5. Average normalized peak area of five sterols (silylation at 70°C). Chole = cholesterol; Campe = campesterol; Stigma = stigmasterol; ß-sito = ß-sitosterol; ß-sitosta = ß-sitostanol.

above, 4 h incubation at 70°C was considered optimal for sterol silylation, reducing the derivatization time by 66% (from 12 to 4 h). Better calibration curves were generated with acceptable linearity and correlation coefficient (Fig. 6). Further reduction in analytical time was possible by modifying GC temperature conditions and holding times, but this had a negative impact on the standard curves and overall linearity. Instrument gas flow was also adjusted for achieving nonoverlapping reproducible peaks with reduced tails.

Quality Assurance and Quality Control (QA/QC)

Field and laboratory blanks were tested, and the sterol extracts were analyzed in duplicate for QA/QC. In addition, some effluent samples were also extracted and analyzed in triplicate. The observed instrument variation was 5 to 8%, while different extracts from the same effluent sample varied about 15%. For treated effluent samples, surrogate recoveries were relatively high (> 92%). However, the recoveries from sludge samples were relatively low, about 70% on average.

Results for reproducibility and precision for two plant sterols, ß-sitosterol and cholesterol, are shown in Fig. 7 and 8. Gas chromatograms for primary effluent (secondary biobasin influent) and biologically treated (secondary or final) effluent are shown in Fig. 9 and Fig. 10, respectively. Phytosterol calibration curves were prepared using eight or more points for each sterol (Fig. 6). The calibration was checked as required, and the instrument was recalibrated when the effluent matrix changed. Identification of each sterol was confirmed by its mass spectrogram. Standard blanks and duplicate samples were used to check analytical variability. The average method detection limit was estimated to be 5 μg/L for individual sterols. However, 8 to 10 μg/L was taken as the practical quantification limit for the extracted sterols. Sterol extracts from treated PPMEs were relatively cleaner as compared with the extracts from secondary sludge.

Fig. 6. Sterols standard curves (silylation, 4 h at 70 °C). Chole = cholesterol; Ergo = ergosterol; Campe = campesterol; Stigma = stigmasterol; ß-Sito = ß-sitosterol; ß-Sitosta = ß-sitostanol.

Fig. 7. Quality assurance/quality control analysis for ß-sitosterol (silylation, 4 h at 70°C).
The Distribution of Plant Sterols in Primary and Secondary Effluents

The modified technique was used for sterols in PPMEs from two British Columbia mills, A and B. The results for five weeks of primary effluent (Mill A) and secondary effluents (Mill B) sampling are shown in Table 1. On average, primary effluents contained 800 ± 190 µg/L total sterols including cholesterol, ergosterol, campesterol, stigmasterol, ß-sitosterol, and ß-sitostanol or stigmastanol. The most abundant sterol was ß-sitosterol, while ergosterol appeared to be the least abundant. Sterols make up about 6.0% of the wood extractives and about 10.6% of the bark extractives of pines (Hafi zoglu 1989). ß-sitosterol is the main sterol found in wood pulps and has been identified in the PPME receiving waters (Cook et al. 1997). The maximum detected concentration of ß-sitosterol was 390 µg/L, or 48% of the total sterols. The average concentrations of ß-sitosterol, ß-sitostanol, and campesterol were 320, 210, and 158 µg/L, which represented about 39, 26, and 20% of the total sterols, respectively (Table 1). Hence, ß-sitosterol, ß-sitostanol, and campesterol collectively accounted for about 80% of the sterols found in primary treated PPMEs from Mill A.

In general, these results appear to be in agreement with the reported literature (Magnus et al. 2000, Mahmood-Khan and Hall 2003). The sterols were present in easily detectable concentrations in PPMEs from local sources. Sterols released from wood during pulping processes may finally show up in PPMEs. Primary treatment of PPMEs may have partially removed the incoming sterols, but a significant portion of the sterols was still present after the primary treatment.

Secondary treated samples were mainly received from Mill B. Most of the sterols present in the primary effluents were also detected in the biologically treated secondary effluents (Table 1). The secondary or final effluents contained 211 ± 90 µg/L total sterols on average. In final effluents, ß-sitostanol was the major sterol, accounting for about 43% of the total sterols, instead of ß-sitosterol which was the major sterol in primary effluents. ß-sitosterol and campesterol followed ß-sitostanol quantitatively (Table 1). Stigmasterol, ergosterol, and cholesterol were present in low concentrations but not

Fig. 8. Quality assurance/quality control analysis for cholesterol (silylation, 4 h at 70°C).

Fig. 9. Chromatogram for a primary effluent sample showing six plant sterols found in PPMEs (silylation, 4 h at 70°C) (Dotriacontane, internal standard).

Fig. 10. Chromatogram for a biologically treated final effluent sample showing four different plant sterols present in PPMEs (silylation, 4 h at 70°C) (Dotriacontane, internal standard).
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in all the final effluent samples. The results demonstrated a variation in the individual sterol distributions for primary and secondary effluents. A possible reason for the observed dissimilarity in the sterol distributions may be the fact that primary effluents came from Mill A and the secondary effluents came from Mill B. A preferential removal of some sterols, particularly β-sitosterol, during secondary treatment may also affect sterol distribution in final effluents.

Plant Sterols and Secondary Wastewater Treatment

The removal efficiencies for individual sterols were not calculated because the primary and secondary PPMEs came from different mills, and the purpose of the study, at this stage, was to focus on the analytical technique that can quantify plant sterols in the primary and secondary treated PPMEs. Nonetheless, average sterol concentrations are given in Table 2 for comparison. If the level of sterols in the primary effluents from Mill B is assumed to be approximately similar to that at Mill A, the lower concentrations in secondary effluents suggest removal of sterols through secondary treatment. However, the extent and mechanism of sterol removal, i.e., biodegradation or removal from the liquid phase, were not clear from these results.

Effective removal of organics from PPMEs (Oikari and Holmbom 1996) and biodegradation of similar contaminants, such as wood resins, through modern activated sludge systems have been shown (Marscheck et al. 1972; Werker 1998). This may suggest some removal of sterols through biodegradation or biotransformation. Other studies suggest sorption of contaminants like sterols onto suspended solids and biomass in the aeration basin (Kaplin et al. 1997; Sundin et al. 1999; Mahmood-Khan and Hall 2003). Biosorption and biodegradation may be important mechanisms for sterol removal from PPMEs in secondary treatment due to sterol sorptive affinity for secondary solids (Kostamo and Kukkonen 2003; Mahmood-Khan and Hall 2003; McKague and Reeve 2003). However, more investigation seemed necessary to establish and evaluate PPME sterol removal through secondary biological treatment.

This study modified a procedure for the reliable analysis of plant sterols, and successfully applied the developed technique to primary and secondary treated effluents for sterol quantification. The technique shall be used in further experiments to investigate possible removal mechanisms of sterol bioadsorption and biodegradation or biotransformation during secondary biological treatment.

Conclusions

A gas chromatographic technique was modified for effective isolation, extraction, and quantification of phytosterols, suspected endocrine disrupting chemicals,
from a complex mixture of PPMEs. The procedure involved liquid-liquid extractions using MTBE, and optimum silylation followed by GC/MS analysis. The developed technique successfully quantified plant sterols in primary and secondary treated effluents.

Optimum silylation was required for effective chromatographic determination of the sterols extracted from PPMEs. Silylation appeared to improve the quality of gas chromatograms, analytical reproducibility, and detection levels. Optimum silylation was achieved by incubating the extracted sterols with BSTFA for about 4 h at an increased temperature of 70°C. The modified conditions reduced the derivatization duration from 12 to 4 h. This reduced the total analytical time required for sterol analysis, and improved method sensitivity for low level sterol concentrations found in treated PPMEs.

Different sterols were quantified in PPMEs collected from two mills. Total sterol content was about 800 ± 190 μg/L in primary treated PPMEs, and about 211 ± 90 μg/L in biologically treated PPMEs or final secondary effluents. β-sitosterol, β-sitostanol, and campesterol comprised the major fraction and accounted for about 80%. Cholesterol, ergosterol, and stigmasterol were also present in relatively lower quantities. A general comparison of plant sterols in primary and secondary treated PPMEs suggested a good removal of about 73% across the sampled biological secondary treatment systems.

Acknowledgments

We wish our thanks to the two British Columbia coastal pulp and paper mills for the provision of PPME sample collection from their treatment facilities and for the delivery of the required effluent samples. Our special thanks to Paula Parkinson and Susan Harper for their analytical assistance during this investigation. We gratefully acknowledge the financial support from the Education Ministry of Pakistan, NSERC Canada, and COMSATS IIT Pakistan.

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<table>
<thead>
<tr>
<th>Effluent stream</th>
<th>Chole</th>
<th>Ergo</th>
<th>Campe</th>
<th>Stigma</th>
<th>β-Sito</th>
<th>β-Sitosta</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary effluents (Mill A)</td>
<td>44.5</td>
<td>26.8</td>
<td>158.5</td>
<td>31.1</td>
<td>319.6</td>
<td>211.4</td>
<td>792</td>
</tr>
<tr>
<td>Secondary effluents (Mill B)</td>
<td>9.4</td>
<td>19.0</td>
<td>26.4</td>
<td>10.3</td>
<td>55.1</td>
<td>90.8</td>
<td>211.1</td>
</tr>
</tbody>
</table>

Approximate removal | 78.88% | 29.1% | 83.34% | 66.88% | 82.76% | 57.05% | 73.35%

*Chole = cholesterol, Ergo = ergosterol; Campe = campesterol; Stigma = stigmasterol; β-Sito = β-sitosterol; β-Sitosta = β-sitostanol.*


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Received: 24 September 2007; accepted: 9 June 2008.
Application of SCR Technology for Degradation of Reactive Yellow Dye in Aqueous Solution

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The effect of sonochemical reactors (SCR) technology upon the degradation of reactive yellow dye has been studied and reported here. Sonochemical reactors (ultrasound irradiation) produce strong cavitation in aqueous solution causing shock wave and reactive free radicals by the violent collapse of the cavitation bubble. These effects should contribute to destruction as well as the decomposition of dyes. This research investigated the efficacy of sonochemical reactors for decolourizing reactive yellow dyes in aqueous solution. In this research, the influence of concentration, frequency, treatment time, and reactor power on the dye decomposition were investigated. The results obtained from the study carried out have shown that SCR can be used effectively for degradation of reactive yellow dyes. The results suggested sonochemical reactors provided maximum destruction, and 120 min treatment time at 130 kHz and 500 W were the most effective for the maximum degradation of reactive yellow dye.

Key words: sonochemical reactor, reactive yellow dye, frequency, treatment time, power

Introduction

In the case of the textile industry, reactive or direct dyes prevail; there is also a large percentage of dispersed dyes. The colour in the water strongly absorbs sunlight, which decreases the intensity of its assimilation by water plants and phytoplankton and reduces the self-purification capacity of water reservoirs. Also, dyes used in the textile industry may be toxic to organisms living in the surface water, and cannot be naturally biodegraded (Trotman 1975; Hunger 2003).

Reactive dyes are commonly used on cotton as they have good wet-fastness, which depends on converting soluble substances into relatively insoluble compounds in the fiber. Reactive dyes have complicated chemical structures, including organic ring forms with colour-giving double bonds. Typical of reactive dyes is the formation of a stable covalent bond between the hydroxyl groups of the cellulose fibers and the reactive groups of the dye (Hendrickx and Boardman 1995; Jiraratananon et al. 2000; Chakraborty et al. 2003).

The traditional technologies for degradation of dye from effluent are the use of activated carbon, filtration, coagulation, and ozone. Each technology has advantages and disadvantages. For example, the use of activated carbon is technically easy but has a high waste disposal cost. But conventional filtration technology will allow for the passage of pure water, and low-molar-mass dyes. Coagulation and sedimentation technology, such as using ferric salts, lime, or alum, is also a low-cost process. However, disposal of the sludge produced is a concern. The ozonation process has a high treatment cost. Also, there are a large number of advanced oxidation processes currently being investigated, which use a sonochemical reactor alone and in combination with ozone or hydrogen peroxide (Hart et al. 1990; Petrier et al. 1992; Stanislaw et al. 2001; Zhou and Smith 2002).

Sonochemical reactor technology is relatively new, and it is simple to use. The technology exposes aqueous solutions containing the organic compounds to ultrasound. The ultrasound wave is possible to improve effectiveness of process compared with the traditional technology by the effect of cavitation. Liquids irradiated with ultrasound can produce bubbles. Once formed, small gas bubbles irradiated with ultrasound will absorb energy from the sound waves and grow. The collapse of these bubbles spawn extreme conditions such as very high temperatures (10,000K) and pressures (up to 10,000 atm), which in turn lead to the dissociation of H2O and the production of ‘OH, HOO’. A local high amount of OH radicals exists at the interface zone of the collapsing bubbles, and then oxidative destruction of the reactive yellow dye quickly occurs by the reaction with OH radicals in this zone. On the other hand, the amount of OH radicals is very high at the effective reaction zone after the bubbles collapse (Thakore 1990; Suslick 1994; Laborde and Bouyer 1998; Okitsu et al. 2005).

The major objective of this study was to investigate and apply sonochemical reactors as a novel treatment technology for degradation of dye from aqueous solution.
Materials and Methods

Apparatus and Procedure

The water solutions of the reactive yellow dye were analyzed by a spectrophotometric method. This method is applicable to potable and surface waters and to wastewater, both domestic and industrial. Measurements were made using a spectrophotometer in the wavelength range at 455 nm by the use of an ultraviolet-visible apparatus (Lambda 25 Perkin Elmer, Sheltom). The filtration system consisted of the following: filtration flasks, 250 mL, with side tubes; Walter crucible holder; filter aid; glass gooch filtering crucible with fritted disk, pore size 40 to 60 micron; and a vacuum apparatus.

An optimal pH was necessary because of the variation of colour with pH. Excessive quantities of suspended materials were removed by centrifuging. Each sample was treated separately, as follows: thoroughly mixed 0.1 g of filter aid in a 10-mL portion of centrifuged sample and filtered it to form a precoat in the filter crucible; directed filtrate to a waste flask; mixed 40 mg of filter aid in a 35-mL portion of centrifuged sample; with the vacuum still on, filtered through the precoat and passed filtrate to a waste flask until clear; then directed clear-filtrate flow to clean flask by means of the three-way stopcock and collected 25 mL for the transmittance determination.

Determination of light transmission characteristics was as follows: thoroughly cleaned 1-cm absorption cells with detergent and rinsed with distilled water; rinsed twice with filtered sample; cleaned external surface with lens paper; filled cell with filtered sample; set instrument to read 100% transmittance on the distilled water blank; and made all determinations with a narrow spectra band.

Reactive dye was dissolved in water to provide an initial absorbance. Aqueous solutions of dye were prepared to give initial concentrations having 1, 2, 3, 4, and 5 mg/L.

Sonochemical Reactor Set-Up

Sonochemical degradation was carried out in a stainless steel reactor, which was operated at 155 and 500 W with an ultrasound generator (Elma TI-H-5, Germany) equipped with two piezoelectric transducers (5-cm diameter) fixed at the bottom of the vessel (Fig. 1). The frequencies of the ultrasound waves generated were 35, 42, and 130 kHz. The reaction temperature was controlled with the help of condensation water surrounding the reactor bath. The characteristics of the reactor are listed in Table 1. All the analyses were performed according to the procedures outlined in standard methods (APHA 1995).

Calculation Method

The definition of reactive yellow dye degradation efficiency is as follows: \( R \) (%) is the degradation efficiency of the sonochemical reactor (SCR), \( C_0 \) is the initial concentration of reactive yellow dye (mg/L), and \( C_t \) is the concentration of reactive yellow dye (mg/L) after reaction for (t) time.

\[
R \% = \left( \frac{C_0 - C_t}{C_0} \right) \times 100
\]

Statistical Analyses

The effect of using SCR technology on the degradation of the reactive dye was analyzed statistically by using SPSS 11.5. The variables were initial concentration, treatment time, frequency, and power. Statistical analyses were carried out using Pearson correlation (Table 2) and multiple linear regression (Table 3). The statistical analyses showed that there were linear relationships between degradation ratio, treatment time, and concentration as shown Table 3.

Results

Aqueous solutions of reactive yellow dyes were sonicated in a batch reactor for different concentrations, power, frequencies, and times. During the sonodegradation, the concentrations of reactive yellow dye were determined and the ultraviolet absorption spectra of the aqueous solution of reactive yellow dye were measured. In order to observe the relative effects of an operation parameter

---

**TABLE 1. Characteristics of SCR used in the experiments**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power</td>
<td>155 W, 500 W</td>
</tr>
<tr>
<td>Frequency</td>
<td>35 kHz, 42 kHz, 130 kHz</td>
</tr>
<tr>
<td>Reactor type</td>
<td>Basin</td>
</tr>
<tr>
<td>Flow type</td>
<td>Batch</td>
</tr>
</tbody>
</table>

---

Fig. 1. Laboratory SCR for degradation of dye.
### TABLE 2. Pearson correlations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Removal</th>
<th>Time</th>
<th>Conc.</th>
<th>Frequency</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearson correlation</td>
<td>.483**</td>
<td>.394**</td>
<td>.554**</td>
<td>.305**</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td>.000</td>
<td>.000</td>
<td>.008</td>
</tr>
<tr>
<td>Time</td>
<td>Pearson correlation</td>
<td>.483**</td>
<td>1</td>
<td>.004</td>
<td>-.026</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td>.972</td>
<td>.827</td>
<td>.684</td>
</tr>
<tr>
<td>Concentration</td>
<td>Pearson correlation</td>
<td>.394**</td>
<td>.004</td>
<td>1</td>
<td>-.152</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.972</td>
<td>.193</td>
<td>.457</td>
<td>.000</td>
</tr>
<tr>
<td>Frequency</td>
<td>Pearson correlation</td>
<td>.554**</td>
<td>-.026</td>
<td>-1.52</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.827</td>
<td>.000</td>
<td>.000</td>
<td>.000</td>
</tr>
<tr>
<td>Power</td>
<td>Pearson correlation</td>
<td>.305**</td>
<td>-.048</td>
<td>-.087</td>
<td>.537**</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.684</td>
<td>.457</td>
<td>.000</td>
<td>.000</td>
</tr>
</tbody>
</table>

*Correlation is significant (Sig.) (**) at the 0.01 level (2-tailed).

^ Conc. = concentration.

### TABLE 3. Multiple linear regression tests

#### A. Variables entered/removed

<table>
<thead>
<tr>
<th>Variables entered</th>
<th>Variables removed</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td></td>
<td>Forward (Criterion: Probability-of-F-to-enter &lt;= .050)</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td>Forward (Criterion: Probability-of-F-to-enter &lt;= .050)</td>
</tr>
<tr>
<td>Concentration</td>
<td></td>
<td>Forward (Criterion: Probability-of-F-to-enter &lt;= .050)</td>
</tr>
</tbody>
</table>

#### B. Model summary

<table>
<thead>
<tr>
<th>Model</th>
<th>(R)</th>
<th>(R^2)</th>
<th>Adjusted (R^2)</th>
<th>Standard error of the estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.554*</td>
<td>.307</td>
<td>.298</td>
<td>88503</td>
</tr>
<tr>
<td>2</td>
<td>.745*</td>
<td>.554</td>
<td>.542</td>
<td>.71476</td>
</tr>
<tr>
<td>3</td>
<td>.888*</td>
<td>.788</td>
<td>.779</td>
<td>.49596</td>
</tr>
</tbody>
</table>

#### C. ANOVA

<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>(F)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Regression</td>
<td>25.361</td>
<td>1</td>
<td>25.361</td>
<td>.000*</td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>57.179</td>
<td>73</td>
<td>.783</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>82.540</td>
<td>74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Regression</td>
<td>45.757</td>
<td>2</td>
<td>22.878</td>
<td>.000 c</td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>36.783</td>
<td>72</td>
<td>.511</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>82.540</td>
<td>74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Regression</td>
<td>65.076</td>
<td>3</td>
<td>21.692</td>
<td>.000 d</td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>17.464</td>
<td>71</td>
<td>.246</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>82.540</td>
<td>74</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### D. Coefficients

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized coefficients</th>
<th>Standardized coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(B)</td>
<td>Standard error</td>
</tr>
<tr>
<td>1</td>
<td>(Constant)</td>
<td>-.066</td>
</tr>
<tr>
<td></td>
<td>Frequency</td>
<td>.014</td>
</tr>
<tr>
<td>2</td>
<td>(Constant)</td>
<td>-.1.241</td>
</tr>
<tr>
<td></td>
<td>Frequency</td>
<td>.015</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>.016</td>
</tr>
<tr>
<td>3</td>
<td>(Constant)</td>
<td>-.2.548</td>
</tr>
<tr>
<td></td>
<td>Frequency</td>
<td>.016</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>.016</td>
</tr>
<tr>
<td></td>
<td>Concentration</td>
<td>.353</td>
</tr>
</tbody>
</table>
on the reactive yellow dye degradation rate during treatment, initial dye concentrations were adjusted to 1, 2, 3, 4, and 5 mg/L before treatment, and aqueous dye concentrations were monitored at various intervals (Table 4).

### Discussion

#### Effect of Concentration

The effect of the concentration of reactive dye used on the rate of treatment was examined. The effect of the initial reactive concentration on removal is shown in Table 5; different initial reactive concentrations resulted in different removal ratios. The degradation ratio increased with increasing initial reactive concentrations in the range of 1 to 5 mg/L under sonication as shown in Table 2. As expected, the degradation rate is the fastest for the highest dye concentration (5 mg/L) applied, and the degradation rate is the lowest for the lowest dye concentration (1 mg/L) applied, equal to 4.05 and 0.91 mg/L, respectively (*p* value < 0.001, *r* = 0.394). On the other hand, correlation is significant at the 0.01 level. As shown in Tables 6 and 7, when initial concentrations were 2 and 5 mg/L, the removal amounts were 0.96 and 1.25 mg/L (42 kHz, 155 W), and the removal amounts were 0.64 and 0.70 mg/L (35 kHz, 500 W) after 120 min, respectively. Clearly, the rate of treatment is high in the presence of high concentrations of dye. On the other hand, the increase of dye concentration in the solutions significantly increased the rate of dye destruction after 120 min.

### Table 4. Experimental conditions for treatment operations

<table>
<thead>
<tr>
<th>Frequency (kHz)</th>
<th>Power (W)</th>
<th>Treatment time (min)</th>
<th>Initial concentration (mg/L)</th>
<th>Temp. (°C)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>130</td>
<td>500</td>
<td>20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120</td>
<td>1, 2, 3, 4, 5</td>
<td>19–20</td>
<td>6.5–7</td>
</tr>
<tr>
<td>42</td>
<td>155</td>
<td>30, 60, 90, 120</td>
<td>2, 5</td>
<td>19–20</td>
<td>6.5–7</td>
</tr>
<tr>
<td>35</td>
<td>500</td>
<td>20, 40, 60, 80, 100, 120</td>
<td>2, 5</td>
<td>19–20</td>
<td>6.5–7</td>
</tr>
</tbody>
</table>

### Table 6. Removal amount (mg/L) of reactive yellow dye at 42 kHz and 155 W

<table>
<thead>
<tr>
<th>Treatment time (min)</th>
<th>Initial reactive dye concentration</th>
<th>2 mg/L</th>
<th>5 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.14</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.28</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>0.44</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>0.96</td>
<td>1.25</td>
<td></td>
</tr>
</tbody>
</table>

### Table 7. Removal amount (mg/L) of reactive yellow dye at 35 kHz and 500 W

<table>
<thead>
<tr>
<th>Treatment time (min)</th>
<th>Initial reactive dye concentration</th>
<th>2 mg/L</th>
<th>5 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.24</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>0.28</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.32</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>0.42</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.46</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>0.64</td>
<td>0.70</td>
<td></td>
</tr>
</tbody>
</table>

#### Effect of Treatment Time

The effect of treatment time on the degradation of reactive dyes was shown in Table 5. Statistical analyses showed that the degradation ratio increased with increasing treatment time (*p* value < 0.001, *r* = 0.483), as shown in

### Table 5. Removal amount (mg/L) of reactive yellow dye at 130 kHz and 500 W for different initial reactive dye concentrations

<table>
<thead>
<tr>
<th>Treatment time (min)</th>
<th>Initial reactive dye concentration</th>
<th>1 mg/L</th>
<th>2 mg/L</th>
<th>3 mg/L</th>
<th>4 mg/L</th>
<th>5 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.30</td>
<td>0.56</td>
<td>0.57</td>
<td>0.68</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.48</td>
<td>0.86</td>
<td>0.99</td>
<td>1.28</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>0.65</td>
<td>1.14</td>
<td>1.44</td>
<td>1.48</td>
<td>1.70</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.67</td>
<td>1.24</td>
<td>1.53</td>
<td>1.80</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.66</td>
<td>1.32</td>
<td>1.70</td>
<td>2.12</td>
<td>2.10</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>0.76</td>
<td>1.46</td>
<td>1.89</td>
<td>2.36</td>
<td>2.85</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>0.85</td>
<td>1.58</td>
<td>2.19</td>
<td>2.88</td>
<td>3.10</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>0.86</td>
<td>1.70</td>
<td>2.37</td>
<td>3.04</td>
<td>3.65</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.88</td>
<td>1.70</td>
<td>2.49</td>
<td>3.24</td>
<td>3.95</td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>0.89</td>
<td>1.74</td>
<td>2.58</td>
<td>3.28</td>
<td>4.00</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>0.91</td>
<td>1.80</td>
<td>2.67</td>
<td>3.40</td>
<td>4.05</td>
<td></td>
</tr>
</tbody>
</table>
A growing degree of solution decolouration was observed. With an increase in the treatment time, a reactive yellow dye with SCR was higher during the 120 min reaction. The results indicated that the degradation ratio of the 0.01 level. The removal amount of dyes in the range of 1 to 5 mg/L was 0.91, 1.80, 2.67, 3.40, and 4.05 mg/L under ultrasonication during 120 min at 130 kHz and 500 W.

However, removal amount of reactive dye in the range of 2 and 5 mg/L was 0.96 and 1.25 mg/L during 120 min at 42 kHz and 155 W. Also, the removal amount was 0.64 and 0.70 mg/L during 120 min at 35 kHz and 500 W. This study showed that treatment time is one of the most important parameters in determining the degradation ratio. The results indicated that the degradation ratio of reactive yellow dye with SCR was higher during the 120 min reaction. With an increase in the treatment time, a growing degree of solution decolouration was observed.

**Effect of Power and Frequency**

The effect of power on dye degradation was also studied, and it was found that the sonodegradation percentage using SCR was almost variable at different powers (500 and 155 W). Comparison of the reactive yellow dye degradation ratio at various frequencies and power was estimated, and efficiencies are presented in Tables 6 and 7; within 42 kHz and 155 W of treatment (120 min), the removal amount was 0.79 and 1.25 mg/L, respectively. Within the 35 kHz and 500 W treatment (120 min), removal amount was 0.64 and 0.70 mg/L, respectively.

The decolouration rate is the highest at the highest applied ultrasound frequency (130 kHz), (p value < 0.001, r = 0.554). On the other hand, correlation is significant at the 0.01 level, as shown in Table 2.

Finally, according to Table 3 D, linear relationship equations are as follows:

- Degradation ratio (Model 1) = -0.066 + 0.014 frequency
- Degradation ratio (Model 2) = -1.241 + 0.015 frequency + 0.016 time
- Degradation ratio (Model 3) = -2.548 + 0.016 frequency + 0.016 time + 0.353 concentration

**Conclusions**

- A sonochemical reactor was used in the treatment of dye solutions under different conditions of operation (e.g., sonochemical time, power, frequency, and initial concentration). These preliminary experiments indicate that a sonochemical reactor has efficacy in the degradation of reactive dye that may be present in solutions.
- The treatment efficiency rose with the increasing dye content of the solution.
- Degradation of reactive yellow dye increased with increasing applied sonication reactor frequency.
- Our statistical analyses showed that ultrasound frequency and treatment time are the most effective parameters for degradation of reactive yellow dye, respectively.

**Acknowledgment**

This research has been supported by Tehran University of Medical Sciences and Health Services, Grant # 2454.

**References**


Received: 10 December 2006; accepted: 14 April 2008.
Factors Influencing Formation of Trihalomethanes in Drinking Water: Results from Multivariate Statistical Investigation of the Ontario Drinking Water Surveillance Program Database

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The presence of trihalomethanes (THMs) in drinking water is an important issue in the context of their potential health effects. Numerous studies have developed models in the past three decades relating THMs concentrations to different factors (e.g., dissolved organic carbon [DOC], chlorine dose, pH, etc.). Previous studies characterized the importance of specific factors through controlled studies using synthetic water or source waters from a small number of water treatment plants. Few studies have reported looking for factors related to THMs formation system-wide across many different water supply systems, and in environments where many factors vary simultaneously. This study presents the results of a multivariate statistical analysis for 162 water supply systems in Ontario, Canada for 2000 to 2004. Principal component analysis (PCA) was applied to determine important factors and possible clusters of variation. PCA identified DOC, chlorine dose, pH, temperature, and reaction time as significant factors for THMs formation. Separate clusters were observed for DOC-colour; chlorine dose-total/free residual chlorine; and hardness-alkalinity. Each cluster indicated factors varying together and representing significant variation. Temperature and pH were found significant and uncorrelated throughout the analysis. The multivariate analysis is the first phase of a continuing investigation into THMs formation with the ultimate goal of developing a predictive model, which can be used to perform human health risk-cost balance studies for drinking water quality management.

Key words: factors for trihalomethanes formation, multivariate statistical analysis, principal component analysis, simultaneous variation

Introduction

The Drinking Water Surveillance Program (DWSP) of the Ministry of the Environment for the Province of Ontario monitors drinking water quality throughout Ontario, Canada. For the period of 2000 to 2004, the DWSP measured different water quality factors from 179 municipal drinking water supply systems (MWSS) throughout Ontario (MOE 2006). More than 90% of these MWSS use chlorine as their primary disinfectant. The reactions between natural organic matter (NOM) and chlorine form disinfection by-products (DBPs) in drinking water, which have several groups, including trihalomethanes (THMs), haloacetic acids (HAAs), haloacetonitriles, and haloketones. Epidemiological studies show that increased bladder, colon, and rectal cancers, as well as acute and chronic effects, such as cardiac anomalies, stillbirths, miscarriages, low birth weights, and preterm deliveries, are associated with waters containing higher levels of DBPs (Cantor et al. 1998; Mills et al. 1998; King et al. 2000). King and Marrett (1996) reported that 14 to 16% of bladder cancers in Ontario, corresponding to approximately 230 to 260 bladder cancer incidents per year, can be attributed to drinking waters containing relatively high levels of DBPs. In Canada, THMs are the only DBP group that is currently regulated; other individual DBPs that are regulated are bromodichloromethane, bromate, and chlorite (Health Canada 2007). Regulations for HAAs are being developed (Health Canada 2007). In order to improve the understanding of human health risk from finished drinking waters, THMs formation has been extensively investigated since being discovered in 1974.

Rodrigues et al. (2007) used fractional factorial experimental designs to investigate THMs formation (assessing NOM as one of the factors) using NOM collected from the Caldeirao dam in Guarda, Portugal. While previously reported investigations have identified pH and reaction time as significant for THMs formation, pH and reaction time were not statistically significant in the Rodrigues et al. study. The experimental design used by Rodrigues et al. (2007) did not include replicates, which could be employed to provide a better estimate of the noise variance for use in assessing statistical significance, and this might have an impact on their conclusions. Rodriguez et al. (2003) predicted THMs formation using multivariate regression models and neural networks for two databases, which were developed from raw waters in nine utilities during 1982 to 1984 and 14 locations of the Mississippi River and its tributaries during 1991 to 1992. The results of Rodriguez et al. (2003) shed light on characteristics of raw water that might have had an influence on THMs formation. However, most of the MWSS currently apply pretreatment prior to chlorination, which substantially changes THMs
formation potentials (MOE 2006). Thus, results for raw water characteristics may not entirely reflect the factors influencing THMs formation after pretreatment. Clark et al. (2001) developed kinetic models for THMs formation using synthetic water. This study performed full three-level factorial designs for three factors (pH, reaction time, and bromide ion concentration), keeping total organic carbon (TOC) and temperature constant; the effects of TOC and temperature were not characterized in their study. Significant numbers of other studies developed models for THMs formation in the past (Rathbun 1996; Amy et al. 1987; Clark and Sivaganesan 1998; Gang et al. 2002; Sohn et al. 2004). However, these studies did not characterize the effects of individual factors, focusing instead on developing a predictive model using the characteristics of the water samples without employing any planned perturbations to consider simultaneous variation of the factors. A number of other studies characterized the effects of different factors on THMs formation (Stevens et al. 1976; Engerholm and Amy 1983; Singer et al. 1995; Summer et al. 1996; White et al. 2003). However, these studies mostly varied one factor by keeping the others constant.

A survey of the reported studies indicates that 22 different variables have been considered for implication in THMs formation: TOC, dissolved organic carbon (DOC), pH, ultraviolet light (absorbance at 254 nm [UV254]), chlorine demand, chlorine dose, free residual chlorine, temperature, reaction time, bromide ion concentration, chlorophyll, THMs formation rate constants, ratio between THMs and chlorine consumed, NVTOC (nonvolatile total organic carbon), dimensionless time in treatment plants and distribution systems, dispersion parameter, ammonia nitrogen, summer/spring seasons, algae, and rapid/slow reaction rate constants (Chowdhury and Champagne 2008). One of the complicating factors in determining significant factors is that some of the factors in this list have been found to be strongly correlated, and some represent similar water characteristics (Chowdhury and Champagne 2008), e.g., TOC, DOC, and UV154. These three factors are surrogate measures of NOM in water. Simultaneous use of more than one of these three variables may introduce confounding effects in models, in which several variables or terms are performing the same role, and can lead to ill-conditioned parameter estimation problems (Montgomery and Runger 2007).

A complementary perspective can be gained, however, by examining how various factors characterizing water quality vary together naturally (e.g., NOM, pH, temperature) and in the MWSS (e.g., chlorine dose, reaction time), and by examining THMs formation in light of simultaneous variation. Natural water characteristics typically follow a pattern that reflects aspects of the water source and natural surroundings. THMs formation is influenced by this natural variation and the subsequent steps taken in the MWSS for treatment. In particular, correlation is likely to exist between chlorine dose and NOM since operators adjust chlorine dose to account for changes in the characteristics of the natural water. Understanding the variation patterns between water characteristics, treatment, and THMs formation on a large scale is the goal of the study. The Ontario DWSP database provides measurements for a number of the factors in the set of 22 identified above, along with measurements of THMs concentrations and measurements of other factors that might be associated with THMs formation. These measurements are provided over a five year period (2000 to 2004) for 179 MWSS in Ontario. The database contains representative numbers of groundwater- and surface water-sourced MWSS.

The properties of source waters in the Ontario DWSP database before and after pretreatment are shown in Table 1. Pretreatment reduces NOM (measured as DOC), which substantially reduces THMs formation. 

| TABLE 1: Physical properties of raw and pretreated water in Ontario*       |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                             | pH              | Alkalinity (mg/L) | Hardness (mg/L) | Colour (TCU)    | Temperature (°C) | DOC (mg/L)      | Turbidity (Formazin TU) |
| Data count                  | 1,381           | 1,423            | 1,392           | 1,267           | 1,383           | 1,427           | 1,351           |
| **Raw water**               |                 |                  |                 |                 |                 |                 |                 |
| Range                       | 6.9–7.5         | 5.5–592          | 9–1,230         | 0.2–150         | 0–28.5          | 0.2–32.8        | 0.02–189        |
| MLV                         | 7.6             | 95.6             | 119             | 9               | 9.4             | 2.7             | 1.08            |
| Mean                        | 7.62            | 128.86           | 161             | 16.3            | 10.4            | 4.12            | 4.79            |
| STD                         | 0.533           | 89.3             | 127.3           | 19.6            | 6.7             | 3.9             | 12.5            |
| **Treated water in the plants** |                 |                  |                 |                 |                 |                 |                 |
| Range                       | 5.74–9.4        | 10–590           | 8–1,250         | 0.2–75          | 0–29.5          | 0.5–20.4        | 0.02–12.25      |
| MLV                         | 7.4             | 86.3             | 119             | 1.8             | 10              | 2.1             | 0.11            |
| Mean                        | 7.4             | 115.7            | 157.8           | 3.6             | 10.8            | 2.6             | 0.24            |
| STD                         | 0.48            | 82.3             | 125.8           | 6.24            | 6.72            | 2.1             | 0.49            |
| Regulatory limits           | 6.5–8.5         | 30–500           | 100             | 5               | 15              | 5               | 1               |

*MLV: Most likely value; STD: Standard deviation.
In treatment plants, a portion of chlorine reacts with reduced substances in the water, such as Fe$^{2+}$, Mn$^{2+}$, and S$^{2-}$ (Gang et al. 2002), while NOM consumes most of the chlorine (Rodriguez et al. 2002). The reactions are further complicated by the presence of ammonia, which can form combined chlorine (e.g., monochloramine, dichloramine) for chlorine to ammonia mole ratios <1 (Connel 1997; MWH 2005). With further addition of chlorine, a fraction of the chloramines is converted into nitrogen trichloride, and the remaining chloramines are oxidized to nitrous oxide and nitrogen, with the chlorine reduced to chloride ions (White 1999). The chlorine utilized by the reduced substances is typically small and not associated with THMs formation (Rodriguez et al. 2002). In this study, correlations among factors and THMs formation were analyzed for the pretreated samples from the Ontario DWSP database using graphical and quantitative (Principal Components Analysis) techniques. The paper begins by describing the DWSP and the characteristics of the water. Correlations and matrix scatterplots are then used to provide a preliminary indication of correlation patterns, and to determine whether some of the codependencies are nonlinear (these might not be detected by correlation which identifies systematic linear relationships). The results of the principal components analysis (PCA) are then summarized, and the conclusions drawn are compared with results from previous controlled studies. Finally, a similar investigation to identify the most important factors for HAAs formation is provided.

### Sampling and Data Statistics

The Ontario Ministry of the Environment has developed sample collection and handling guidelines which were followed by the DWSP to collect and preserve water samples (MOE 2003). Grab sampling methods were used by the DWSP in accordance with the Drinking Water System Regulation (DWSR 2003). The samples from raw/treated water and distribution systems were collected on a regular basis and analyzed in laboratories of the Ministry of the Environment and the Ministry of Labour in Ontario. The raw water samples were collected prior to the treatment, typically from the intake points. The raw waters are generally pretreated using one or more of the following physical/chemical treatment processes (screening, coagulation/loculation, mixing, settling, and filtration) to reduce DBPs precursors, turbidity, and pathogens (Edzwald 1993; Chowdhury et al. 2007). For treated waters, samples were collected from treatment plants after pretreatment, and, for the groundwater-sourced plants, raw water samples were collected from individual wells, while the treated water samples were collected from reservoirs. The samples from the distribution systems were collected to account for elevated storage tanks, dead ends, ageing water mains, distribution loops, cross connections/back flows, chlorine booster stations, and extremities of the distribution systems. The samples were collected headspace-free using 40-mL EPA type glass vials or 250-mL glass bottles in duplicate and preserved in refrigerators in the dark at 5 ± 3°C before analyses. Sodium thiosulphate was used to preserve the samples. The samples were analyzed within 14 days of collection following standard analytical protocols (APHA 1995; MOE 2001, 2003; DWSR 2003). The methods, which are used by the Ontario Safe Drinking Water Act following regulation 169/170, were followed in the laboratory analyses (LSB 2003; DWSR 2003). In determining the THMs and DOC, the LSB (Laboratory Services Branch) followed OPOV-E3144 and ROM-E3370 methods, respectively.

The DWSP survey included factors that might be associated with THMs formation, and provided an indication of THMs variability for 179 MWSS. A number of MWSS (Ear Falls, Fauquier, Smith Falls, Bourget Well Supply, Clarence Creek Well Supply, Norwich, Dunnville, Bayside, Innisfil, and Walpole Island) reported high levels of chlorine doses (10.2 to 53.7 mg/L), while the chlorine doses for the bulk of the treatment plants were much less (less than 10.1 mg/L). The high chlorine levels could be due to specific needs of those MWSS to produce the required free residual chlorine. In addition, some MWSS (St. Pascal, Ottawa Britannia, Hearst, Ottawa Lemieux Island, Bourget, Clarence Creek, Chapleu, Mattice, and Sault Ste. Marie) use postchloramination. The other MWSS use chlorinone (chlorine gas, liquid sodium/calcium hypochloride) to disinfect water prior to distribution. The formation of THMs in the plants and distribution systems using chloramine may be different than the others using chlorine. The remaining 162 MWSS were analyzed in this study.

The chlorine dose, total chlorine, combined chlorine, and free residual chlorine, as well as THMs concentrations for the 162 MWSS are shown in Table 2. The mean chlorine dose for the 162 MWSS was calculated to be 2.64 mg/L with a range of 0.14 to 10.1 mg/L. The THMs concentrations varied between 0.5 to 273 μg/L with a mean of 31.6 μg/L in the water treatment plants, while the mean THMs concentration was observed higher in the water distribution systems (40.2 μg/L) with a range of 0.5 to 289 μg/L. The data show that 80 to 90% of THMs were formed in the treatment plants (TP), while 10 to 20% was formed in distribution systems (DS). The averages of total chlorine were 1.32 and 0.81 mg/L in the TP and DS, respectively. The free residual chlorine had a mean of 0.98 mg/L in the TP and 0.54 mg/L in the DS, while the combined chlorine had a mean of 0.38 mg/L in the TP and 0.31 mg/L in the DS (Table 2). The DOC in the raw water had a mean value of 4.1 mg/L, while it was 2.6 mg/L for treated waters; DOC has been noted as the main precursor of THMs and HAAs formation (Rodriguez et al. 2003; Sohn et al. 2004). The statistical distributions of THMs, DOC, chlorine dose, pH, total chlorine, and temperature are shown in Fig. 1. The distributions for pH and total chlorine were found to be relatively symmetric, while those of the remaining variables were generally asymmetric with long upper and lower tails. The outliers in Fig. 1 were excluded from the analyses to avoid possible biasing effects.
Chowdhury et al.

Fig. 1. Possible outliers for treatment plant data by box-plots (surface water).

Multivariate Analyses

Pairwise correlations and scatterplots. Although subsets of the 22 different variables listed earlier have been previously used in the modelling of THMs formation, the DWSP database did not include each of these 22 variables. After careful review of previous models, correlation patterns, and available measurements, the following twelve variables were identified as having possible influence on THMs formation: chlorine dose, total chlorine, combined chlorine, free residual chlorine, pH, temperature, turbidity, alkalinity, colour, hardness, DOC, and dissolved inorganic carbon (DIC). The combined chlorine is the difference between total chlorine and free residual chlorine, and alkalinity in natural water is mostly contributed by the bicarbonates, which is essentially the amount of total DIC (APHA 1995). Consequently, combined chlorine and DIC were omitted from the analyses.

In this study, correlation analyses for the ten remaining variables (DOC, colour, pH, alkalinity, hardness, turbidity, temperature, chlorine dose, total chlorine, and free residual chlorine) were performed using data from the water treatment plants. The data were analyzed in two groups: (1) surface water-sourced plants, and (2) groundwater-sourced plants. All computations were performed using the JMP statistical package.

Correlation analyses: surface water-sourced plants. The correlations amongst the variables are summarized in Table 3. The chlorine dose had a weak correlation with total chlorine, and a strong correlation with free residual chlorine ($r = 0.31$ and $0.78$, respectively). The DOC had a moderate correlation with colour ($r = 0.55$), and alkalinity had a strong correlation with hardness ($r = 0.84$). The formation of THMs had statistically significant correlations with DOC, colour, pH, temperature, total chlorine, and chlorine dose (Table 3). However, chlorine dose, total chlorine, and free residual chlorine arise from the same phenomenon, namely chlorination. As such, it is important to identify the most representative variable and its correlation with the other variables of this group. Some variables in this group were correlated: for instance, chlorine dose and free residual chlorine (Table 3). The correlations of THMs with turbidity, alkalinity, and hardness were not statistically significant (Table 3). Considering chlorine dose from the chlorine group and DOC, colour, pH, and temperature, the pairwise comparisons are summarized in Table 4 and indicate strong to modest correlations between THMs and DOC ($r = 0.78$), THMs-chlorine dose ($r = 0.59$), and THMs-colour ($r = 0.48$). Relatively weaker correlations were observed between THMs and pH ($r = 0.24$) and THMs and temperature ($r = 0.16$). From these data, DOC was found to be correlated with chlorine dose ($r = 0.66$) and colour ($r = 0.56$). Figure 2 depicts correlations through a scatterplot matrix, which assisted in the identification of nonlinear codependencies. Plots of THMs to DOC; DOC to chlorine dose; and DOC to colour were found to have a systematic linear trend in Fig. 2. The correlation between DOC and chlorine dose likely reflects the operating policies of the MWSS. As DOC increases, operators are likely to add more chlorine to ensure disinfection and appropriate free residual chlorine. This correlation reflects the “feedback” present in the operating data.

Correlation analyses: groundwater-sourced plants.

The groundwater correlation analysis presented
Applying PCA on Factors for THMs Formation

slightly different correlations than those of the surface water-sourced plants. Chlorine dose was found to be moderately correlated with total chlorine \( (r = 0.43) \) and strongly correlated with free residual chlorine \( (r = 0.91) \), while hardness was well correlated with alkalinity \( (r = 0.7) \) and DOC and colour were moderately correlated \( (r = 0.63) \). The correlation pattern between the factors and THMs were: DOC \( (r = 0.57) \), chlorine dose \( (r = 0.55) \), pH \( (r = 0.31) \), temperature \( (r = 0.2) \), and colour \( (r = 0.13) \). In groundwater data, the correlation of DOC with THMs \( (r = 0.57) \) was smaller than that of the surface water data \( (r = 0.78) \). The pH was been found to have slightly higher correlation with THMs in groundwater \( (r = 0.31) \) than that of the surface water data. However, the correlation between DOC and colour was found to be fairly consistent in all cases (groundwater \( [r = 0.63] \) and surface water \( [r = 0.56] \) data). Chlorine dose was selected for further analysis. The correlation analysis for THMs is summarized in Table 5. The scatterplot matrix for these variables is shown in Fig. 3. The DOC-colour and

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**TABLE 3. Multivariate correlations for surface water-sourced data in treatment plants**

<table>
<thead>
<tr>
<th></th>
<th>Cl&lt;sub&gt;2&lt;/sub&gt; dose</th>
<th>Cl&lt;sub&gt;2&lt;/sub&gt; total</th>
<th>Cl&lt;sub&gt;2&lt;/sub&gt; free</th>
<th>pH</th>
<th>Temp.</th>
<th>Turbidity</th>
<th>Alkalinity</th>
<th>Colour</th>
<th>Hardness</th>
<th>DOC</th>
<th>THMs</th>
</tr>
</thead>
<tbody>
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<td>Cl&lt;sub&gt;2&lt;/sub&gt; dose</td>
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<td>0.25</td>
<td>-0.04</td>
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<td>-0.11</td>
<td>0.14</td>
<td>0.13</td>
<td>0.36</td>
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<tr>
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<td>-0.06</td>
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<td>0.16</td>
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<tr>
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<td>-0.18</td>
<td>0.01</td>
<td>0.1</td>
<td>0.08</td>
</tr>
<tr>
<td>pH</td>
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<td>-0.09</td>
<td>-0.14</td>
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<td>0.06</td>
<td>-0.14</td>
<td>0.18</td>
<td>-0.16</td>
<td>0.08</td>
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<td>Colour</td>
<td>-0.11</td>
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<td>0.18</td>
<td>-0.05</td>
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<td>Hardness</td>
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<td>0.01</td>
<td>0.84</td>
<td>0.08</td>
<td>1</td>
<td>0.02</td>
<td>-0.07</td>
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<td>DOC</td>
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<td>0.13</td>
<td>0.1</td>
<td>0.08</td>
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<td>0.16</td>
<td>0.09</td>
<td>-0.03</td>
<td>0.39</td>
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<td>0.74</td>
<td>1</td>
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**TABLE 4. Pairwise correlations for six variables in treatment plant (surface water-sourced data)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>by Variable</th>
<th>Correlation</th>
<th>Significant probability</th>
<th>Plot Correlation</th>
</tr>
</thead>
<tbody>
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<td>pH</td>
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<td>Colour</td>
<td>Temperature</td>
<td>0.0298</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>DOC</td>
<td>pH</td>
<td>-0.0516</td>
<td>0.3096</td>
<td></td>
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<tr>
<td>DOC</td>
<td>Temperature</td>
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<td>Colour</td>
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<td>DOC</td>
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<td></td>
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</table>

Fig. 2. Scatter plot matrix for parameters (surface water-sourced data).
THMs-DOC pairs were found to exhibit similar trends as found in surface water data (Fig. 3). Moderate to weak correlations were noted for the THMs-DOC dose, THMs-chlorine dose, DOC-colour dose, colour-chlorine dose, and DOC-chlorine dose (Table 5). The results obtained from repeating the pairwise comparison with the inclusion of possible outliers indicated that DOC was correlated with THMs ($r = 0.61$) and chlorine dose ($r = 0.4$). The correlation for the groundwater data were fairly similar to those obtained for the same pairs in the analysis of the surface water data. Temperature and pH had positive effects on THMs formation in both cases, which was in agreement with the previous studies.

### Table 5. Pairwise correlations for groundwater-sourced data in treatment plant

<table>
<thead>
<tr>
<th>Variable</th>
<th>by Variable</th>
<th>Correlation</th>
<th>Significant probability</th>
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<td>THMs</td>
<td>Temp</td>
<td>0.2005</td>
<td>0.0349</td>
</tr>
<tr>
<td>THMs</td>
<td>Colour</td>
<td>0.1281</td>
<td>0.1822</td>
</tr>
<tr>
<td>THMs</td>
<td>DOC</td>
<td>0.5655</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

**Principal component analysis (PCA).** PCA is a multivariate statistical technique that decomposes the correlation structure of the data, leading to the identification of patterns and contrasts in datasets (Jackson 1991). PCA transforms correlated variables into new uncorrelated variables by defining principal components. By examining the original variables associated with a principal component, patterns of variation in which a subset of the original variables take values in a systematic pattern can be identified. PCA also helps to reduce dimensionality of data, allowing the bulk of the variation in the data to be represented using a smaller number of transformed variables (Wold et al. 1987; Jackson 1991). In recent years, PCA has been applied in water quality data analyses, bioremediation, and environmental monitoring (Bengrane and Marhaba 2003; Praus 2005; Hon et al. 2006; Mrklas et al. 2006; Rao et al. 2006). PCA is usually applied by first mean-centering the data and scaling each variable by its standard deviation (Wold et al. 1987; Smith 2002). The PCA calculation itself can be considered as either computing singular value decomposition (SVD) on the scaled and centered data matrix, or computing eigenvector decomposition on the $X^T X$ matrix, where $X$ is the data matrix (Jackson 1991). When the scaling has been performed, the eigenvalues represent the fraction of total variation explained by the principal component (Jackson 1991). Intuitively, the PCA algorithm can be considered as follows: for $n$ original variables, $n$ principal components are formed by: (i) finding the linear combination (principal component) of the original variables explaining the greatest possible variance; and (ii) successively finding each principal component having the greatest possible variance that is uncorrelated (orthogonal) with all previously defined components. This restriction on correlation with the previous components ensures that each new component

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**Fig. 3.** Scatter plot matrix for parameters (groundwater data analysis).
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will have a lower variance than its predecessor. The linear combinations that make up a principal component are found from the elements of the eigenvectors from the eigenvector decomposition.

The loadings in PCA represent the extent to which each original variable contributes to the principal component. The loading values themselves correspond to the elements in the eigenvectors from the eigenvector decomposition. The correlation structure in the data can be analyzed by examining the patterns of the loadings and identifying clusters of the original variables that contribute to specific principal components. The loadings can be summarized graphically by using bar charts to represent the loading values for a single principal component (Fig. 4), and by plotting pairs of principal component loadings against each other (Fig. 5). The bars in Fig. 4 identify the original variables that contributed significantly to the given principal component, while the scatterplots (Fig. 5) identify clustering of the original variables in the principal components. A threshold value of 0.4 was used to judge whether a principal component loading was large or not. This threshold is somewhat arbitrary; more rigorous thresholds could be developed using statistical arguments, but the threshold of 0.4 suffices to distinguish between larger and smaller loading magnitudes. In this study, PCA was applied to the surface water and groundwater data separately. For each set of data, ten variables were analyzed: (1) chlorine dose; (2) total chlorine; (3) free residual chlorine; (4) pH; (5) temperature; (6) turbidity; (7) alkalinity; (8) colour; (9) hardness; and (10) DOC. The variables were scaled and mean-centered prior to the application of PCA. The statistical package JMP was used to perform the PCA computations.

**Loadings of variables: surface water-sourced plants.** From Figure 4a(i), it can be noted that chlorine dose (1), total chlorine (2), free residual chlorine (3), colour (8), and DOC (10) contributed significantly to the first principal component (PC1). Since these variables were associated with colour/DOC and chlorine dose, PC1 was related to the organic contents of the water. Principal component 2 (PC2) was primarily associated with chlorine dose (Fig. 4a[ii]). Principal component 3 (PC3) had significant loadings for pH (4) and alkalinity (7), while temperature (5) and hardness (9) also had loadings close to 0.4 (Fig. 4a[iii]). Thus, PC3 is associated with the inorganic characteristics of the water (acidity/alkalinity, hardness,
and inorganic carbon), as well as temperature. Pairwise scatterplots of the loadings can be examined to identify clusters of variables explaining significant components of variability. The plot of PC1 versus PC2 loadings in Fig. 5a(i) confirmed that chlorine dose (1) was significantly associated with both PC1 and PC2. On the other hand, total chlorine (2), free residual chlorine (3), colour (8), and DOC (10) were associated with PC1 only. Because of this clustering, it can be concluded that PC1 was associated with chlorination and organic contents, while PC2 was associated with chlorine dose only. This suggested that chlorination varied significantly in MWSS across Ontario, associated in part with organic contents in the water. The plot of PC1 versus principal component 3 (PC3) (Figure 5a[ii]) indicated clear distinction between variables associated with PC1 and PC3. PC1 was associated with chlorine dose (1), free residual chlorine (3), and DOC (10), with loadings for total chlorine (2) and colour (8) lying very close to the threshold value of 0.4. Consequently, PC1 for groundwater-sourced data was comparable to PC1 for surface water-sourced data, and it can be concluded that PC1 for each case corresponds to organic content and chlorination. PC2 for the groundwater case differed from PC2 for the surface water case, with significant loadings identified for chlorine dose (1), free residual chlorine (3), pH (4), temperature (5), and DOC (10), compared with chlorine dose (1) only for surface water (Fig. 4b[i], 4a[ii]). Hence, for groundwater sources PC2 was associated with chlorination, acidity, temperature, and organic contents of the water. PC3 for groundwater also differed from its counterpart for surface water, with significant loadings for temperature (5), and loadings for pH (4) and colour (8) close to the threshold value (Fig. 4b[iii]). The types of significant loadings for PC3 in the groundwater case were still associated with inorganic properties (acidity, alkalinity, hardness) and temperature of water. It is anticipated that one or two variables from each cluster may be required for modelling THMs formation.

Loadings of variables: groundwater-sourced plants.

Figures 4b and 5b summarize the PC loadings for the groundwater data. As shown in Fig. 4b(i), the significant loadings for PC1 were chlorine dose (1), free residual chlorine (3), and DOC (10), with loadings for total chlorine (2) and colour (8) lying very close to the threshold value of 0.4. Consequently, PC1 for groundwater-sourced data was comparable to PC1 for surface water-sourced data, and it can be concluded that PC1 for each case corresponds to organic content and chlorination. PC2 for the groundwater case differed from PC2 for the surface water case, with significant loadings identified for chlorine dose (1), free residual chlorine (3), pH (4), temperature (5), and DOC (10), compared with chlorine dose (1) only for surface water (Fig. 4b[i], 4a[ii]). Hence, for groundwater sources PC2 was associated with chlorination, acidity, temperature, and organic contents of the water. PC3 for groundwater also differed from its counterpart for surface water, with significant loading for temperature (5), and loadings for pH (4) and colour (8) close to the threshold value (Fig. 4b[iii]). The types of significant loadings for PC3 in the groundwater case were still associated with inorganic properties (acidity, alkalinity, hardness) and temperature of water. It is anticipated that one or two variables from each cluster may be required for modelling THMs formation.
of the water (e.g., mineral content), or whether organic contents were also associated with PC3 for groundwater. Given that other factors associated with organic contents (in particular, chlorination-related variables) were not significant, it was possible that colour in groundwater could be associated with inorganic contents.

The clustering of loadings between PC1 and PC2 for groundwater (Fig. 5b[i]) was much looser than the corresponding clustering for surface water (Fig. 5a[i]). This could be partly due to the fewer observations available for groundwater MWSS compared with surface water observations, which constitute the largest part of the DWSP database. However, there were also distinct differences in the loading pattern for PC2 between the surface and groundwater cases. In ground waters, chlorine dose (1), total chlorine (2), free residual chlorine (3), colour (8), and DOC (10) contributed significantly to PC1 and, as noted above, PC1 was associated with organic contents and chlorination. Chlorination also appeared with a significant loading in PC2, through loadings for chlorine dose (1) and free residual chlorine (3), along with pH (4), temperature (5), and DOC (10). In the groundwater case, PC1 was associated with organic contents and chlorination, while PC2 was associated with organic content, chlorination, acidity, and temperature. In particular, free residual chlorine (3) and DOC (10) stood out in Fig. 5b(i) as having significant loadings for both PC1 and PC2.

The loadings plot of PC1 versus PC3 (Fig. 5b[iii]) indicates that PC1 was associated with chlorine dose (1), total chlorine (2), free residual chlorine (3), colour (8), and DOC (10). PC3 was associated with temperature (5), pH (4), and colour (8). The plot of PC2 versus PC3 (Fig. 5b[iv]) shows that pH (4) and temperature (5) played significant roles in both PC2 and PC3, in contrast to the clear separation of loadings observed between PC2 and PC3 for the surface water case. PC2 was associated with chlorine dose (1), free residual chlorine (3), pH (4), temperature (5), and DOC (10), while PC3 was associated with pH (4), temperature (5), and colour (8). Summarizing, for the groundwater-sourced MWSS, PC1 represented organic contents and chlorination, PC2 represented organic contents, acidity, chlorination, and temperature, while PC3 represented temperature and inorganic properties of the water. The primary difference between the correlation patterns for surface water and groundwater-sourced MWSS was in PC2, which for surface water was associated with chlorination, while in the groundwater case it was associated with chlorination, organic content, acidity, and temperature. The analyses of the DWSP data sets by PCA showed that approximately 80% of the total variability of data was represented by the first three principal components.

Discussion

The DWSP data were reported from regular monitoring for five years (2000 to 2004) of 179 MWSS in Ontario (Canada). The variability in the DWSP data has made it difficult to identify patterns through a simple examination of individual constituent values and locations. To obtain a more definitive picture of the significant variables in a MWSS that might have an effect on THMs formation, it was necessary to perform a multivariate statistical investigation of these data. Although ten variables were analyzed from the DWSP dataset, only a few of these variables were found to be significant, and not all of the variables were found to be independent. Insights regarding how physical characteristics occur together were obtained by analyzing the correlation structure in the DWSP dataset using pairwise correlations, scatterplots, and PCA. The DOC was found to have a strong correlation with THMs concentrations, with correlation coefficients of 0.78 and 0.57 respectively for surface water and groundwater data. This was in agreement with results obtained in previous studies (Singer and Chang 1989). Colour and DOC were found to be correlated (r = 0.56 to 0.63) and clustered throughout the analyses. The correlation between chlorine dose and free residual chlorine varied throughout the analyses, but was found to be strong (0.78 to 0.91). The THMs concentrations were found to have significant correlations with chlorine dose (0.55 to 0.59). For waters that are pretreated, the concentrations of reduced substances are generally insignificant, and thus chlorine dose can be a significant factor for THMs formation. Free residual chlorine generally varied in the range of 0.03 to 3.05 mg/L in Ontario (MOE 2006), and this free residual chlorine was found to have an insignificant effect on THMs formation (Table 3).

Temperature had significant loading for PC3 in surface water data and for PC2 and PC3 in groundwater data, and was found to be correlated with THMs throughout the analyses. The correlations of temperature with THMs were weak (r = 0.12 to 0.2) but statistically significant (Table 4 and Table 5, p < 0.05). The pH was found to have significant loading on PC3 of the surface water and groundwater data, and on PC2 for the groundwater data. The pH was found to be correlated with THMs (r = 0.21 to 0.31) throughout the analyses (Tables 4 to 5), which was also statistically significant (Table 4 and Table 5, p < 0.05). Stevens et al. (1976) performed bench-scale experiments on Ohio River water at the Cincinnati treatment plant by varying temperature and pH respectively. The formation of THMs was found to be 1.5 to 2 times higher at each stage of temperature change (3 to 25 and 25 to 40°C). Similar findings were noted for pH change (30 to 50% increase of THMs while pH was changed from 7 to 11. However, Stevens et al. (1976) did not incorporate simultaneous variability of other factors during their experiments, for example, as to whether the impact of pH on THMs formation would be different at different values of temperature or DOC.

In the natural systems, factors such as DOC and chlorine content vary temporarily and spatially in addition to pH and temperature. The analyses summarized in this paper have investigated correlation patterns amongst many factors that might influence THMs formation.
in a natural environment. In the multivariate analyses presented here, the effects of DOC, chlorine dose, pH, temperature, and colour were simultaneously determined. It is likely that DOC and chlorine dose have the highest effects on THMs formation. The pH and temperature are the next important factors as determined throughout the analyses. The concentrations of bromide ion were not available in the DWSP data and, thus, an analysis of the effect of bromide ion concentrations could not be presented here. The presence of bromide ions in chlorinated water typically leads to the increased formation of brominated THMs (Nokes et al. 1999). Due to the potential concerns of brominated THMs on human health, it would be important to study the effects of bromide ions on brominated THMs. In this study, separate investigations were performed to determine the effects of different factors on HAAs formation; for these investigations, the same input factors were considered. With the exception of pH, all other factors have similar effects on HAAs formation. At higher pH, HAAs show decreasing trends, which may be attributed to hydrolysis of many halogenated DBPs at higher pH (Krasner et al. 1989; Singer 1994).

Conclusions

Disinfection for drinking water is a requirement for MWSS in Ontario. However, the formation of DBPs is of concern because of their possible effects on human health. The Ontario DWSP data, collected over a period of five years (2000 to 2004), were analyzed in this study using pairwise correlations, matrix scatterplots, and PCA. Important variables were identified, and the sensitivity of the results to possible outliers has been investigated. The DWSP data analyses identified DOC and chlorine dose as the factors having the strongest association with THMs formation, with pH and temperature having significant but relatively less association with THMs formation. The THMs in the distribution systems were found to be higher than those of the treatment plants, as anticipated, indicating possible effects of reaction time on THMs formation through slow reactions with the residual organics in the water distribution pipes. For the surface water data, the three largest principal components, accounting for 80% of the variability in the dataset, were associated with a) chlorination and organic content, b) chlorination, and c) inorganic properties (acidity/alkalinity, hardness) and temperature. For the groundwater data, the three largest principal components, accounting for 76% of the variability in the dataset, were associated with a) chlorination and organic content, b) organic content, acidity, chlorination, and temperature, and c) inorganic properties (acidity/alkalinity and hardness) and temperature. The insight gained in this analysis has formed the basis for an experimental investigation of factors influencing THMs formation using statistical design of experiments approaches.

Acknowledgments

The financial support of the Natural Sciences and Engineering Research Council of Canada in the form of a Canada Graduate Scholarship, and Queen’s University in the form of a Queen’s Graduate Award, is gratefully acknowledged. The cooperation of Dave Fellowes, Ministry of Environment, Ontario (Canada) for the provision of the DWSP data is gratefully acknowledged.

References


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Received: 7 February 2008; accepted: 9 June 2008.
The Effect of Short-Term Dissolved Oxygen Transients on Activated Sludge

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The effect of short-term dissolved oxygen (DO) disturbances on municipal activated sludge was studied, in a batch system, with respect to changes in supernatant turbidity, suspended solid (SS) concentrations, proteins, polysaccharides, and cations in the extracellular polymeric substances (EPS). Results showed that turbidity increased by 20 times when the DO concentration decreased below 0.5 mg/L, and supernatant SS concentrations increased by 1 to 2 times with DO reduction, implying the presence of more unsettled particles in the supernatant. Concomitantly, soluble proteins increased from less than 1 mg/L to up to 30 mg/L, and bound proteins decreased by more than 15% under DO limitation. Further enzymatic tests confirmed that, compared with polysaccharides, proteins were more involved in preventing sludge deflocculation. The DO stress also caused significant changes in the bulk concentrations of K+ and Ca2+; K+ increased by 40% and Ca2+ decreased by 30%. When the DO concentration was restored after 6 hours, reversible changes were observed in supernatant turbidity and SS, and concentrations of EPS proteins and cations, indicating a possible physiological response of microorganisms to a short-term low DO disturbance.

Key words: activated sludge, deflocculation, dissolved oxygen transient, proteins, polysaccharides, cations

Introduction

Biological wastewater treatment plants are subject to numerous variations and steady-state conditions are seldom present (Daigger and Grady 1982). Such unsteady-state conditions (i.e., transients) often directly affect solid-liquid separation, imposing undesirable biochemical oxygen demand/chemical oxygen demand (BOD/COD) removal rates, biosolid losses, and toxicant carry-over. Among these transients, low dissolved oxygen (DO) concentration, arising from BOD overloading or insufficient aeration, is a common disturbance and has received increasing attention in recent studies (Starkey and Karr 1984; Berthouex and Fan 1986; Wilén and Balmér 1998, 1999; Wilén et al. 2000a, 2000b; Archibald and Young 2004).

A short-term (hours) transient low DO level has a different impact from a long-term (days or weeks) chronic low DO level in an aerobic reactor. It has been known that filamentous bulking is one of the consequences of long-term DO limitations (Jenkins et al. 1993; Rittmann and McCarty 2001). In contrast, studies on a short-term DO shortage demonstrated deflocculation of biosolids. In an experiment with a continuous activated sludge process performed by Starkey and Karr (1984), increasing supernatant turbidity was observed after 20 hours of operation with shutting off aeration (DO < 1 mg/L). After aeration was restored, a decline in turbidity was observed. Wilén and coworkers (Wilén and Balmér 1998, 1999; Wilén et al. 2000a, 2000b) studied the effect of short-term DO limitation (3 to 4 hours of N2 purging) on activated sludge and observed a similar increase in turbidity. Wilén and Balmér (1998) proposed that disintegration of bioflocs, i.e., deflocculation, could be the cause for increasing turbidity. Currently, turbidity and concentrations of suspended solids (SS) in supernatant or treated effluent are the main parameters to characterize sludge deflocculation phenomenon.

The positive role of extracellular polymeric substances (EPS) in flocculation of bioflocs has been previously recognized (Bura et al. 1998; Liss 2002). Bioflocs are composed of various microbial cells connected by EPS and inorganic particles. Proteins and polysaccharides have been identified as the dominant EPS constituents (FrOlund et al. 1996; Durmaz and Sanin 2001). Depending on the origin of wastewater, humic substances may also be a main EPS component. Fleming and Wingender (2001) suggested that proteins mainly played a functional role, whereas polysaccharides tended to play a structural role in EPS. Proteins provide an active site for cell aggregation in brewer’s yeast (Calleja 1987; Ferreira et al. 1994). In biofilm development, proteins play a significant role in initial attachment to abiotic surfaces, as well as the formation of a mature biofilm (O’Toole and Kolter 1998; Danese et al. 2000).

EPS structure has been proposed to be two different layers in a floc matrix (Gehr and Henry 1983; Jorand et al. 1995; Laspidou and Rittmann 2002): (i) bound EPS refer to those that are tightly bound to cells or in cell vicinity, and are difficult to extract; (ii) soluble EPS are those that are loosely embedded in the floc matrix or on the surface of bioflocs, and are relatively easy to extract. Since limited investigations have been conducted on investigating the changes in proteins and polysaccharides...
upon DO disturbances, we attempted to examine the proteins and polysaccharides in bound and soluble EPS layers in the present study.

Apart from the EPS, the importance of cations in microbial flocculation has been previously emphasized (Zita and Hermansson 1994; Sobek and Higgins 2002). It is known that divalent cations (e.g., Ca^{2+} and Mg^{2+}) are involved in sludge flocculation. These cations connect the negatively charged functional groups in the EPS biopolymers. The replacement of divalent ions by monovalent cations could deteriorate the floc strength and cause sludge deflocculation (Higgins and Novak 1997; Murthy and Novak 2001; Bott and Love 2002).

The objective of this study was to identify transient effects of short-term DO deficiency in a batch system by monitoring the changes in supernatant turbidity and SS concentration, and levels of proteins, polysaccharides, and extracellular cations in the biofloc matrix.

**Methodology**

The experiments were conducted using fresh mixed liquor samples from the North Toronto Treatment Plant. Approximately 40,000 m^{3}/day of wastewater is treated by the plant. During the sampling period, the average BOD_{5} in untreated influents and treated effluents were 120 mg/L and less than 5 mg/L, respectively. Total kjeldahl nitrogen in influents varied from 16 to 35.9 mg/L with an average value of 28.8 mg/L. Total phosphorus in influents varied from 1.9 to 7.3 mg/L with an average number of 3.8 mg/L. The average sludge retention time was 6 days. The mixed liquor sample was collected from the end of the aeration tanks and was transported to the laboratory within 45 min.

Experiments on the DO transients were carried out in four, 2-L parallel batch reactors over a period of 12 months. The effective working volume of each reactor was 1.7 L. The operating temperature in each reactor was maintained approximately at 25 to 26°C through a water jacket coupled to an on-off thermal controller. Mixing and aeration in the reactors were provided by magnetic stirring bars and stone diffusers, respectively. One of the reactors was a control reactor with aeration at approximately 160 mL/min from an air pump. The other three were triplicate transient reactors with an arrangement to purge N_{2} at approximately 150 to 175 mL/min from a compressed N_{2} cylinder. The onset of aeration and purging was recorded as time 0. The overall reaction time was 10 hours. The transient reactors were continuously purged with N_{2}, for the first 6 hours, followed by re-aeration from air pumps (160 mL/min) in the remaining 4 hours. Concentrations of mixed liquor suspended solids in the reactors were approximately 2,100 to 2,200 mg/L throughout the experiments. The mixed liquor samples were taken every 2 hours and analyzed immediately after the sampling.

At each sampling time, at least 180 mL of mixed liquor sample was taken from each reactor for measuring turbidity, and soluble and bound EPS as well as bulk cations. Supernatant turbidity was measured as absorbance at 650 nm after 30 mL of mixed liquor sample was centrifuged at 2,000 rpm for 2 min (Wilén et al. 2000a). As mentioned previously, soluble EPS are the portion that is easy to extract. Forty millilitres of mixed liquor was taken for centrifugation at 10,000 x g and 4°C for 15 min (Higgins and Novak 1997). The supernatant was regarded as “soluble” EPS solution for analyzing the soluble EPS components (proteins and polysaccharides) and levels of bulk cations. The modified Lowry method (Hartree 1972; Frölund et al. 1995) was used for protein measurement with bovine serum albumen as a standard. Polysaccharide was measured as glucose equivalent using the anthrone method (Raunkjaer et al. 1994). For the measurement of bulk cations (K^{+}, Na^{+}, Ca^{2+}, and Mg^{2+}), the supernatants were filtered by 0.45-μm syringe filters before being analyzed using inductively coupled plasma-atomic emission spectroscopy (ICP-AES). For the measurement of bound EPS, 110 mL of mixed liquor sample was extracted by the cation exchange resin method (at 1,000 rpm for 1 hour) (Frölund et al. 1996). Then, the sample was centrifuged at 12,000 x g and 4°C for 15 min. The supernatant was taken for analyzing bound EPS components (proteins and polysaccharides).

In addition, at t = 6 hours and 10 hours, 80 mL of mixed liquor sample from each reactor was taken for measuring concentrations of supernatant SS. The mixed liquor sample was first settled for 30 min. The SS concentrations in the supernatant were analyzed according to the Standard Methods (APHA 1995).

A further examination was conducted to understand whether the released soluble biopolymers could re-associate with the bulk Ca^{2+} under the DO transient condition. One milliliitre of soluble biopolymers solution was acidified with 1 mL of concentrated HNO_{3}, and heated under 100°C for 30 min. By this treatment, the biopolymer-associated cations were released for analysis by ICP-AES (Bott and Love 2002).

Although an increase in pH (from 7.5 to 8.5) was observed under the DO limitation, separate experiments showed that pH did not have a significant impact on the above parameters measured in this study.

Enzymatic tests were conducted to examine the importance of the role of proteins and polysaccharides in sludge flocculation. Sludge samples were treated with trypsin, cellulase, and amylase, respectively, and were incubated for 15 min to 8 hours. Turbidity was measured at different times. As a common proteolytic enzyme that degrades proteins, 2 mL of Trypsin (Sigma-Aldrich Co. T-4549, 10x) was added to 18 mL of mixed liquor sample. Nine milligrams of cellulase (Sigma-Aldrich Com. C-1184) was added to 30 mL of mixed liquor sample for degradation of polysaccharides. Since municipal activated sludge from a starch-rich environment was used, amylase (Sigma-Aldrich Co. A-3176) was also attempted at a concentration of 1 mg of amylase in 30 mL of mixed liquor.
Results

Seven independent batch experiments were repeated on different days to confirm reproducibility of the experiments. All the data demonstrated good reproducibility. In each experiment, the control data were measured in duplicate from the control reactor (i.e., duplicate samples were taken at the same time from the control reactor). In contrast, the triplicate transient samples were taken from the three independent transient reactors. A paired two-sample \( t \)-test was used to statistically compare the data sets of control and transient samples. A cutoff \( p \) value = 0.05 was applied for statistical significance.

DO Impact on Activated Sludge

In the transient reactors, a sudden drop in DO concentrations (down to 0.2 mg/L) occurred within 2 min after the onset of \( \text{N}_2 \) purging. The DO levels were maintained below 0.5 mg/L for 6 hours. After the 6th hour, the DO level was restored to above 5.0 mg/L by stopping \( \text{N}_2 \) purging and turning on aeration.

A low DO concentration caused a significant increase in the supernatant turbidity (Fig. 1). After 2 hours of \( \text{N}_2 \) purging, the turbidity of the transient samples was approximately ten times higher than the control. Turbidity continuously increased under oxygen limitation. To further explore causes for increasing turbidity, control and transient supernatant samples were observed under a microscope (Fig. 2). It was found that the transient sample had much more suspended materials than the control, indicating that more cells or small flocs were suspended into the supernatant as a consequence of deflocculation, thus increasing supernatant turbidity (Fig. 2).

When aeration was restored in the transient reactors, a decrease in turbidity was observed (Fig. 1). After 6 hours, the DO concentration in the transient reactors was elevated to above 5.0 mg/L. Supernatant turbidity declined, as shown by the decreasing absorbance in Fig. 1. Less suspended materials were observed under the microscope, indicating a possible reflocculation when the DO stress was removed.

In addition to higher turbidity, the average SS concentration in transient supernatant samples (approximately 160 mg/L) was more than double that in control (approximately 65 mg/L) at the end of the \( \text{N}_2 \) purging phase (\( t = 6 \) hours) (Fig. 3). The high transient SS concentration indicated that more particles did not settle down under low DO. The SS concentration in transient supernatants decreased after re-aeration. The results confirm that deflocculation occurred under DO shortage, and reflocculation occurred when DO stress was removed.

Significant increases in the levels of soluble proteins were observed under DO limitation (Fig. 4). Throughout the experiment, the concentration of soluble proteins in

![Fig. 1. Typical turbidity changes under low DO: From \( t = 2 \) to 6 hours, turbidity in transient supernatant was significantly higher than that in control (\( p = 4.0 \times 10^{-2} \)). The error bars are \( +/- \) one standard deviation of turbidity measurements.](image)

![Fig. 2. Microscopic observation of supernatant: (a) control; (b) transient. The images were taken by Axiovert 200, inverted microscope. The bar represents 50 \( \mu \)m.](image)
control samples remained low and stable (around zero). In contrast, soluble proteins in transient samples increased under the low DO. After 6 hours of N₂ purging, soluble proteins in transient samples reached up to 30 mg/L. In the last 4 hours \((t = 6\) to 10 hours\), upon reintroduction of oxygen, levels of soluble proteins in transient samples were similar to control samples.

From \(t = 2\) to 6 hours, concentrations of bound proteins in transient samples \((112\) to 55 mg/L\) were significantly lower than in control samples \((143\) to 107 mg/L\) (Fig. 5). The decreasing level of bound proteins under DO disturbance suggests that EPS proteins became solubilized during the process.

In contrast to proteins, soluble polysaccharides under DO disturbance were not significantly different from the control (Fig. 6). Also, soluble polysaccharides increased with time even after re-aeration. This indicated that, unlike soluble proteins, the changes in soluble polysaccharides were not reversible upon reintroduction of oxygen. No significant changes in bound polysaccharides were observed between control and transient samples throughout the experiments.

Concentrations of bulk K⁺ and Ca²⁺ changed significantly during DO transient (Fig. 7 and 8). Before starting the experiments, mixed liquor samples contained approximately 9 mg/L of K⁺, 75 mg/L of Na⁺, 55 mg/L of Ca²⁺, and 18 mg/L of Mg²⁺ in bulk phase. During 6-hour DO limitation, the bulk liquid contained more K⁺ \((40\%\) higher) and less Ca²⁺ \((20\) to 30\% lower\). When the DO concentration was restored in the last 4 hours, the concentrations of bulk K⁺ and Ca²⁺ returned close to those in control samples, suggesting reversible changes in the levels of K⁺ and Ca²⁺ under the DO transient. In comparison, no significant changes in Na⁺ and Mg²⁺ were observed under DO limitation.

A negligible amount of extracellular bulk Ca²⁺ was re-associated with soluble EPS under DO limitation (Fig. 9). Through acidification of soluble EPS, all cations embedded in the biopolymer matrix were released. Since insignificant changes in the Ca²⁺ level occurred before and after the acidification, a negligible amount of Ca²⁺ was recombined with soluble biopolymers under DO limitation. In other words, the decreasing levels of bulk Ca²⁺ under DO limitation were not attributed to the re-association with soluble EPS.

The Roles of Proteins and Polysaccharides in Preventing Sludge Deflocculation

Since proteins and polysaccharides are the dominant EPS components in activated sludge, further enzymatic tests were performed to identify their roles in preventing sludge deflocculation. After 20 min, turbidity in a trypsin-treated sample was 4 times higher than the control, and after 2 hours, turbidity in the trypsin-treated sludge was 6 times higher than that without trypsin (Fig. 10). Under the microscope, more suspended materials were observed from the sample treated with trypsin (Fig. 11). Since
trypsin degrades proteins, this demonstrated that proteins were a key factor in preventing sludge deflocculation. In addition, compared with previous results from DO limitation (0 to 6 hours), the increasing turbidity caused by trypsin had a similar magnitude as that under the DO limitation in the first 4 hours (Fig. 10). Thus, it is reasonable to speculate that deflocculation under DO limitation could be related to changes in proteins.

Through a similar test on examining the role of polysaccharides in the biofloc matrix, it was shown that polysaccharides were less involved in preventing sludge deflocculation (Fig. 10). Cellulase and amylase were used to degrade polysaccharides. After 20 min to 8 hours, changes in the turbidity of the sludge treated with cellulase or amylase were negligible.

Additionally, the results from this study support the hypothesis that proteins act as a glue-like component to hold biopolymers in the EPS. Concentrations of proteins and polysaccharides were analyzed in each enzymatic test. Not only proteins were released, a high level of polysaccharides was also obtained after the sample was treated with trypsin. By comparison, a negligible extra amount of protein was present in the sample treated with amylase or cellulase.
Discussion

Results from the present experiments showed that a transient of DO would cause reversible changes in supernatant turbidity, SS concentration, proteins, and ionic strength. All of these were either the consequence or the cause of sludge deflocculation.

Deflocculation and reflocculation were clearly demonstrated by the reversible changes in supernatant turbidity under the DO variations. A short-term reduction (within 6 hours) in DO concentration induced a significant increase in the supernatant turbidity. The microscopic observations suggested that the release of suspended cells and small flocs was the cause for the increasing turbidity. Once DO stress was removed, the turbidity immediately decreased, indicating reflocculation. The reversible change in the turbidity indicated that cell lysis did not occur under short-term DO limitation. However, there was some evidence to suggest that the process was not fully reversible since the transient turbidity did not return all the way to the control after air restoration (Fig. 1). Under the conditions of repeated aerobic and anaerobic cycles, Wilén et al. (2000b) also pointed out that deflocculation slowly became irreversible as indicated by an accumulation of nonflocculated materials.

As a consequence of deflocculation, there were significant increases in soluble EPS proteins under the short-term low DO. Batch experimental results showed a 20-fold increase in soluble proteins under the DO limitation. Soluble proteins were measured from the supernatant after a centrifugation at 10,000 x g. There were no microbial cells observed in the supernatant. This suggested that the increases in soluble proteins were mainly from the EPS matrix rather than from inside the cells. A short-term DO limitation appeared to weaken the floc, allowing more proteins in the EPS matrix to be easily extracted. Thus, an increase in soluble EPS proteins was another indicator of deflocculation. The increases in soluble EPS compounds have not been described in previous studies on short-term DO transients. Wilén et al. (2000b) examined soluble proteins, but did not observe significant changes under DO limitation. Besides DO stress, Murthy and Novak (2001) and Park (2002) also found that a high level of monovalent ions promoted the amounts of soluble proteins as a result of deterioration of bioflocs.

The profiles of increasing turbidity and soluble proteins under DO limitation indicate that deflocculation may be an erosion-like process, removing the surface particles of the biofloc matrix. Previous studies proposed that bioflocs contain a dense inner layer surrounded by a loose outer layer (Eriksson and Alm 1991; Liao et al. 2002; Sheng et al. 2005). In this study, the turbidity profile (Fig. 1) shows that a sharp increase in turbidity occurred in the first 4 hours. From \( t = 4 \) to 6 hours, the transient turbidity tended to plateau. This implied that the deflocculation could occur in the outer layer of bioflocs by removing the loosely attached particles. After 4 hours of deflocculation, the process of removing particles approached the inner compact layer. As a consequence, less particles were sloughed off and the changes in turbidity slowed down. The loss of the shell layer of bioflocs by deflocculation led to the increasing of soluble proteins, for the soluble EPS compounds were mainly located in the shell layer. Collectively, these results suggest that deflocculation under short-term low DO can be an erosion process.

Future work is required for supporting the notion of deflocculation as an erosion process. Particle size distribution is a key parameter in monitoring changes in floc structure under disturbances. Measurement of floc strength can also provide evidence for an erosion process, provided that the dense inner layer and loose outer layer of bioflocs possess different floc strengths. Examinations of the changes in both particle size distribution and floc strength under short-term DO transients are underway.
Bound proteins are critical to maintain the floc’s integrity. A decrease in bound proteins would be correlated to an increase in soluble proteins, provided that bound biopolymers are hydrolyzed to soluble ones. From the study on ionic disturbance, Higgins and Novak (1997) proposed that a decreasing concentration of bound proteins was associated with sludge deflocculation. In their experiments, a significant decrease in bound proteins and high effluent SS concentrations were observed under a disturbance of high Na⁺ (20 mM). With the addition of divalent ions (e.g., Ca²⁺, Mg²⁺), the authors reported that concentrations of bound proteins increased and ESS concentrations decreased. Similarly, in this study, a decrease in bound proteins was observed in conjunction with deflocculation under short-term low DO. The specific cause of this phenomenon is unknown; however, it could be due to activation by specific enzymes or through a structural modification induced by a shift in the physicochemical environment (e.g., ionic strength).

From the current experimental results, the impact of DO limitation on polysaccharides was different from proteins. Both control and transient samples had a similar increasing rate of soluble polysaccharides with time. Since no extra feeds to the reactors were provided throughout the experiments, soluble polysaccharides were presumably produced to serve as a food substrate to the microorganisms.

The changes in bulk Ca²⁺ under DO stress provide an understanding of the mechanism of deflocculation. Since the EPS components, especially proteins, have many negatively charged functional groups, positive divalent ions could bind with the negatively charged groups to form a stable connection in the floc matrix. In this study, it was found that DO stress caused a reduction in extracellular bulk Ca²⁺. Further tests suggested that the reduction of bulk Ca²⁺ was probably attributed to uptake of Ca²⁺ by microbial cells under DO stress, rather than a re-association between soluble biopolymers and Ca²⁺ in the suspension. Accordingly, the reduction of bulk Ca²⁺ under DO stress led to less Ca²⁺ binding sites being available, and weakened the strength of the biofloc matrix.

Apart from bulk Ca²⁺, the release of K⁺ into the extracellular solution was also observed under DO stress, similar to the study on toxin transients by Bott and Love (2002). In their study, a 100% increase of extracellular K⁺ in the soluble solution was observed after a shockloading of N-ethylmaleimide. By comparison, a more than 40% increase in extracellular K⁺ occurred under DO limitation. The released K⁺ could replace the binding sites provided by divalent ions, and the sludge is prone to deflocculation. Overall, the ratio of Ca²⁺ to K⁺ in bulk solution decreased from 6.2 to 2.5 under the DO limitation.

The release of K⁺ and uptake of Ca²⁺ under oxygen variations is different from changes of K⁺ and Mg²⁺ in the process of enhanced biological phosphorus removal (EBPR). K⁺ and Mg²⁺ are essential counterions of polyphosphates in living cells due to their stable bonding with polyphosphates (Kortstee et al. 2000; Schönborn et al. 2001). In the EBPR process, orthophosphate is released in an anaerobic cycle through the degradation of intracellular polyphosphates. In the following aerobic cycle, extracellular phosphate molecules are taken up for generating energy and forming polyphosphates in cells. Simultaneously with the release and uptake of phosphates, K⁺ and Mg²⁺ are released and taken up through the degradation and production of polyphosphates. Different from the EBPR process, the present study on oxygen limitation demonstrated that there was no significant change in Mg²⁺ in the liquid phase and that the changes in the levels of K⁺ and Ca²⁺ were in the opposite direction. Accordingly, mechanisms for cation responses to short-term oxygen limitation are different with the cation changes in the EBPR process.

The enzymatic tests demonstrated that proteins were more important than polysaccharides in preventing sludge deflocculation. Much higher turbidity was obtained when the sample was treated with trypsin, as well as a high level of proteins and polysaccharides released into supernatant. In contrast, there were insignificant changes in turbidity, and a low level of proteins was released when the sludge was treated with amylase and cellulase. These observations are consistent with the floc model described by Higgins and Novak (1997); most polysaccharide molecules connect with proteins, and the proteins are further tightly attached to the cells. Adding a polysaccharide-degrading enzyme will presumably not affect the connection between proteins and cells, whereas, a protein-degrading enzyme will break the bridge between proteins and cells, releasing proteins and polysaccharides into the solution. Overall, these results suggest that proteins are glue-like biopolymers that are key to holding bioflocs together.

Currently, at a molecular level, what triggers deflocculation under DO stress remains unclear. One hypothesis is that specific enzymes are excreted or activated as a stress response to low DO. Under oxygen deficiency, the cells may release or activate specific enzymes to degrade EPS proteins in an attempt to separate from each other and move away from the undesirable environment. As a consequence, the level of glue-like EPS proteins is reduced and the bioflocs are subject to deflocculation. In regards to the changes in extracellular Ca²⁺ and K⁺, another hypothesis is proposed for sludge deflocculation: cells are able to adjust their extracellular ion concentrations via specific membrane-bound ion transporters in responding to oxygen limitation, as shown by the increases in the concentrations of bulk K⁺ and the decreases in the levels of bulk Ca²⁺ under DO limitation. Studies on mammalian cells show that oxygen shortage affects the function of membrane-bound ion channels (e.g., Na⁺–K⁺ ATP-dependent pump, Na⁺–Ca²⁺ exchanger), inducing changes in both intra- and extracellular ion levels (Haddad and Jiang 1993; Jiang and Haddad 1994; Barneo et al. 2004). Despite the complexity of diverse microbial consortia in activated sludge, studies from mammalian cells should
provide insight on understanding microbial responses to a DO disturbance. Collectively, further investigation on release or activation of enzymes, as well as cation responses to DO transients are underway, in an attempt to enhance an in-depth understanding of deflocculation under short-term DO transients.

Mechanisms for responding to transients of short-term low DO are different from those in an anoxic/anaerobic selectors, anaerobic tanks in the EBPR process, and denitrification zones in activated sludge systems. Different microorganisms are involved in the designed unaerated zones compared with those involved in deflocculation under short-term low DO. As aforementioned, it is suggested that deflocculation due to short-term DO limitation mainly occurs on the surface or shell layer of bioflocs. Microorganisms involved in deflocculation are those sensitive to DO levels and are primarily located in the shell layer. Denitrifiers for denitrification are also present in bioflocs, but are usually located near the centre of bioflocs where the DO level is minimal. In an anoxic or anaerobic selector, the growth of specific flocc-forming bacteria is stimulated, and the growth of specific filamentous bacteria is minimized. In other words, only those organisms having a high substrate uptake rate and a high storage capacity are selected (Jenkins et al. 1993). In the EBPR process, a specific group of organisms, known as polyphosphate accumulating organisms, are involved in the removal of phosphate by degrading polyphosphate in an anaerobic cycle (in an anaerobic tank prior to an aeration basin) and taking up orthophosphate in the following aerobic cycle. Therefore, different microbes play a role in various processes and produce different responses to DO limitation.

The presence of other electron acceptors, such as nitrate, nitrite, and sulphate, affects the responses to DO limitation. Wilén et al. (2000a) reported that addition of nitrate alleviated deflocculation under 3 hours of N₂-purging, as shown by turbidity being 1-fold higher than that in control (aerobic) samples, but 50% lower than that in N₂-purging samples without nitrate. Thus, the presence of alternative electron acceptors could mediate the stress responses to short-term DO limitation. On the other hand, if sulphate is used as an electron acceptor, sulphide produced from the reduction of sulphate can react with iron in bioflocs and deteriorate floc stability. In this study, amounts of nitrate and sulphate in mixed liquor were not examined. It is unclear so far whether the identified DO impacts result from anaerobic conditions (i.e., free of oxygen and alternative electron acceptors) or from anoxic conditions (i.e., using NO₃⁻ or SO₄²⁻ as an electron acceptor). Additionally, besides measuring DO levels in the system, redox potential is a general parameter to qualitatively describe the availability of electron acceptors in the system and should be considered in future work.

Continuous experiments are required for better representing the fate of activated sludge under DO disturbances in industry. Different operating configurations (batch or continuous) may affect the observations (Sobeck and Higgins 2002). It is necessary to carry out continuous experiments to mimic the actual operating environment in industry. In the present batch study, there was neither substrate nor nutrients fed through the experiments. The changes in soluble polysaccharides in this study (Fig. 6) suggest that microorganisms may undergo starvation and exhibit different responses compared with DO limitation. Thus, identifying the impacts of short-term low DO in a continuous system is required for generalizing DO effects on activated sludge.

Conclusions

Transient effects of short-term low DO concentrations on municipal activated sludge were studied in a batch system, with respect to changes in turbidity, SS concentration, levels of proteins, polysaccharides, and cations in the biofloc matrix. The main conclusions arising from the study are as follows:

1. A transient of short-term (a few hours) low DO causes deflocculation of activated sludge, as indicated by a high turbidity and a high concentration of SS in the supernatant.
2. Deflocculation under short-term low DO can be an erosion-like process.
3. Compared with polysaccharides, proteins are more important in preventing sludge deflocculation. Low DO leads to a decrease in bound proteins and an increase in soluble proteins.
4. A DO transient causes a significant decrease in Ca²⁺ but a substantial increase of K⁺ in the bulk liquid.
5. Evidence shows that responses of microorganisms to a short-term low DO are a physiological response, as shown by reversible changes in supernatant turbidity and SS, as well as concentrations of proteins and cations (i.e., Ca²⁺ and K⁺).

Acknowledgments

The authors would like to acknowledge the financial support from the Consortium “Minimizing the Impact of Pulp and Paper Mill Discharges” at the Pulp & Paper Centre, University of Toronto; Aracruz Celulose S.A., Carter Holt Harvey Pulp & Paper, Domtar Inc., Eka Chemical Inc., ERCO Worldwide, Georgia-Pacific Corporation, Irving Pulp and Paper Limited, Japan Carlit Co. Ltd., Tembec Inc., and Votorantim Celulose e Papel. This work is also funded by the Natural Sciences and Engineering Research Council (NSERC) of Canada through Post Graduate Scholarship (PGSB) and Ontario Graduate Scholarship (OGS). The authors would like to thank the North Toronto Treatment Plant for assisting in collection of mixed liquor samples, and Pat McVey and Peter Lister for providing operating data about the plant. The authors also thank Kevork Hacat for polysaccharide measurements.
References


Zhang and Allen


Received: 6 June 2006; accepted: 4 April 2008.
The paper presents aspects related to the performance of an anoxic biotrickling filter designed for hydrogen sulphide (H\textsubscript{2}S) removal from biogas. In this process, nitrate was supplied through a nutrient solution as an electron acceptor for anoxic growth of H\textsubscript{2}S-oxydizing microorganisms. The biotrickling filter's packing media consisted of a layer of plastic fibres over volcanic rocks in a ratio 0.78:1 by volume. The total volume of packing media was 0.014 m\textsuperscript{3}. Several H\textsubscript{2}S loading rates (IL) were tested under continuous dynamic conditions, ranging between 20 and 550 g of H\textsubscript{2}S feed/(m\textsuperscript{3}bed·day). Maximum process performance (>95%) was observed for IL ranging up to approximately 300 g of H\textsubscript{2}S feed/(m\textsuperscript{3}bed·day). The degradation of hydrogen sulphide occurred with the formation of both sulphate and elemental sulphur, their formation ratio being dependent on H\textsubscript{2}S loading rate. Elemental sulphur was found to be the dominant degradation product, particularly at IL > 96.18 g of H\textsubscript{2}S feed/(m\textsuperscript{3}bed·day). The use of two biotrickling filters in series was also tested, and a significant improvement in process performance was observed. This technology allows simple operation with low maintenance and has the potential for sulphur recovery.

Key words: anoxic, biofiltration, biogas, biotrickling, denitrification, hydrogen sulphide

Introduction

Hydrogen sulphide (H\textsubscript{2}S) in biogas represents an environmental and safety concern, and its presence in fuels may cause technical problems in the operation of engines (Syed et al. 2006). Due to these aspects, hydrogen sulphide is recognised as a significant barrier in anaerobic digester biogas utilization at municipal sewage treatment plant facilities (Soreanu et al. 2005). Hydrogen sulphide is listed as one of the top five substances released to the environment in Canada as reported in the National Pollutant Release Inventory (NPRI 2002) administered by Environment Canada. While expensive conventional physical-chemical technologies for H\textsubscript{2}S removal from gas fluxes are available (Syed et al. 2006), recent studies show that hydrogen sulphide in biogas can be removed in cost-effective anoxic biological systems by denitrifying bacteria (Soreanu et al. 2007, 2008). For example, \textit{Thiobacillus denitrificans} can metabolize inorganic compounds, such as hydrogen sulphide under anoxic conditions, when nitrate is used as an electron acceptor (Tiedje 1988; Prescott et al. 2002). The process does not affect the methane content in biogas. While nitrate was found to be more efficient in biological H\textsubscript{2}S removal, nitrite was shown to sustain the biological process at a lower performance. Other aspects related to this process, including microbiological investigations, influence of individual operational parameters, and kinetic and degradation rates (etc.), are presented in Soreanu et al. (2007, 2008). Some aspects related to the cost benefits and environmental significance of implementing such a technology in the municipal or industrial sector are also presented in Soreanu et al. (2008).

The present study confirms the previously reported results and shows that the denitrifying microorganisms are developed under specific environmental conditions, which are important in the management and start-up of the biotrickling filter. The influence of H\textsubscript{2}S loading rate on the process performance and the degradation pathway are presented in the present study, as well as other technical aspects such as the use of two biotrickling filters in series for performance enhancement and sulphur recovery.

Material and Methods

Experimental Installation

The experimental installation (Fig. 1) consisted of a biotrickling filter made from a 15-cm diameter polyvinyl chloride column. The column was packed with media consisting of an upper bed of plastic fibre layers (2-cm thickness; 0.1- to 0.2-mm fibre diameter) and a lower bed of volcanic rock (1- to 2.5-cm diameter) in a ratio of 0.78:1 by volume. The total height of the packing media was 0.8 m and the corresponding total volume was 0.014 m\textsuperscript{3}. The packing media was pre-inoculated with a nutrient solution from another anoxic biotrickling filter treating hydrogen sulphide in biogas. The biogas containing hydrogen sulphide was continuously fed at the bottom of the column, while the nutrient solution was continuously fed at the top, countercurrent to the direction of the gas stream. The biogas was generated by a pilot-scale anaerobic digester treating municipal organic waste from Toronto, Ontario, Canada. The nitrified
Effluent from a pilot-scale sequencing batch reactor treating municipal sewage on site was used as the source of nutrient solution. Additionally, the H$_2$S concentration in the biogas was supplemented, when required, using a H$_2$S generation unit. The biogas and the nutrient solution flowrates were measured using a flowmeter (Cole-Parmer, Model PMRI-010296) and an auto-control peristaltic pump (MasterFlex, Model 77200-62), respectively.

Figure 2 shows the simplified diagram of the experimental installation used for the testing of two bioreactors in series. The second reactor in this set-up was identical to the reactor shown in Fig. 1, while the first reactor contained 0.012 m$^3$ of plastic fibres (versus 0.014 m$^3$ of plastic fibres and volcanic rocks in the second reactor). The output biogas flux exiting from the top of the first reactor was fed to the second reactor at the bottom.

Nutrient Solution

The nitrate rich nutrient solution was prepared according to Soreanu et al. (2008). Sodium nitrate (NaNO$_3$) was added in excess in order to raise the initial concentration to 1,400 mg of N-NO$_3^-$ per litre, and thus assure nitrate nonlimiting conditions for long-term testing under variable H$_2$S loading rates. After an initial pH adjustment of the nutrient solution to approximately 6.5 (Soreanu et al. 2008), the pH was itself maintained in the range of 5.8 to 6.3 over the course of the test period. Composition of the nutrient solution was recorded daily. During each experiment, the nutrient solution composition varied due to the nitrate consumption and the formation of the degradation products, however nitrate concentration remained in excess over stoichiometric conditions (Soreanu et al. 2008). The standard deviation of the nutrient solution for the analyzed compounds (presented below) in the samples collected at the same time was less than 5%.

Analytical / Measurements Methods

A gas chromatograph equipped with a thermal conductivity detector (GC/TCD, Agilent 3000A, model G280) was coupled to gas sampling ports located before and after the bioreactor, in order to perform the analysis of biogas for H$_2$S, CH$_4$, CO$_2$, O$_2$, and N$_2$ (Soreanu et al. 2008). The nutrient solution was analyzed for N-NO$_3^-$, N-NO$_2^-$, N-NH$_4^+$, SO$_4^{2-}$, and S$_2$O$_3^{2-}$, according to Standard Methods for the Examination of Water and Wastewater 21st Edition (APHA et al. 2005), using a Dionex ICS 2000 Ion Chromatograph (Method 4110B) and Technicon TRAACS Autoanalyser (Method 4500-NH$_3$ G), respectively, as described in Soreanu et al. (2008). Characterization of the solid material collected from the packing media was performed. Additionally, sulphur, iron, and sodium were extracted from the solid samples using a modified Standard Method 3050 B and analyzed via an inductively coupled plasma optical emission spectrometer (ICP-OES, Perkin Elmer OPTIMA 5300DV) according to Method 6010 B (U.S. EPA SW-846 1996). The pH of the nutrient solution was monitored continuously with an immersed Orion pH electrode (model 912600) coupled to a digital pH controller (ETATRON DS, model PBX0922110BA). The packing bed temperature and the pressure drop across the biofilter were measured as described in Soreanu et al. (2008).

Traces of S$_2$O$_3^{2-}$ and N-NH$_4^+$ were rarely detected in the nutrient solution, thus reference to their presence is neglected in further discussions. Nitrogen could not be
accurately analyzed during the study due to its very small concentration (a mass balance was instead adopted). The temperature gradient and change in pressure across the biofilter were insignificant and are not discussed further.

Experimental Methodology

Experiments were carried out under dynamic conditions for approximately 2 months, where the microorganisms’ acclimatisation time was approximately 5 to 6 days, and the time frame of each test was 3 to 4 days. Over the course of the test period, the biogas was fed continuously at the bottom of the column and the nutrient solution at the top, counter-current to the gas flow, at a constant nutrient solution flow rate of 30 L/h. The influence of H2S loading rate on process performance and on the degradation pathway was investigated under nonlimiting nitrate conditions. H2S loading rate was increased gradually between 24.05 and 543.96 g of H2S feed/(m³bed·day), by varying the biogas flow rate and H2S concentration, while the corresponding gas contact time varied between 12 and 85 minutes. The criteria used for the estimation of process performance were H2S removal efficiency (RE, %), H2S loading rate [IL, g of H2S feed/(m³bed·day)]; or IL’, g of H2S feed per day], elimination capacity [EC, g of H2S removed/(m³bed·day)]; or EC’, g of H2S removed per day] and nitrate demand (g of N-NO3 consumed per g of H2S removed). The formulas for determining these criteria are presented in Soreanu et al. (2008). Theoretical reactions describing possible degradation pathways for hydrogen sulphide oxidation to elemental sulphur and sulphate based on nitrate reduction to nitrite and nitrogen are presented in Soreanu et al. (2008). Hydrogen sulphide, nitrate, sulphate, and nitrite, were quantitatively analyzed in the present study, while elemental sulphur and nitrogen were calculated from the corresponding mass balance.

Result and Discussion

Influence of the H2S Loading Rate (IL)

Figure 3 presents H2S removal efficiency (RE) and the corresponding reactor elimination capacity (EC) under different H2S loading rate (IL) conditions over 30 days of operation. A series of tests were undertaken at different H2S loading rates. For each H2S loading rate, the test was maintained at steady-state for a period of at least 2 days, and the corresponding EC was observed. After each test, the level of H2S was returned to the normal level found in the pilot-scale digester biogas used for this set of experiments.

After the initial start-up of the biotrickling filter, a short acclimatisation period of 4 days (day 0 to 4) was required in order for the bioreactor to reach maximum performance (RE = 100%) (Fig. 3).

As can be observed in Fig. 3, the increase of EC is directly proportional to the increase of IL up to approximately 300 g of H2S/(m³bed·day), and a maximum process performance (RE = 95 to 100%) was recorded as well. When the IL was increased further, no significant improvement in EC was observed, and therefore the RE decreased, [i.e., down to 70.71% for an IL of 543.96 g of H2S/(m³bed·day)]. The decrease in the RE could be mainly attributed to the limited mass transfer between the phases involved in the process (biogas, nutrient solution, biofilm) as a result of their insufficient contact time due to the high IL, as also explained in Soreanu et al. (2007) and Devinny et al. (1999). Increasing the contact time by increasing the packing volume showed significant improvement in process performance as described later in this paper. Other authors (McComas and Sublette 2001; Kim et al. 2002) reported the possible inhibition of biological process by high IL and elemental sulphur accumulation.

Fig 3. Variation of H2S removal efficiency (RE), H2S loading rate (IL), and biofilter elimination capacity (EC) as a function of time.
The variation of EC as a function of IL is plotted in Fig. 4 (curve 1). For comparison, this graph also includes the representation of the results obtained in a previous experiment carried out under similar experimental conditions (curve 2). Only the type of packing media was somewhat different in these studies (plastic fibres in Soreanu et al. 2007 versus plastic fibres plus volcanic rocks in the present study). The straight line corresponds to the maximum theoretical H2S removal efficiency (RE = 100% and IL = EC). Good correspondence between IL and EC was obtained for a small IL range [up to 300 g of H2S/(m3bed·day)], while for the highest IL range, EC tended to stabilize around 350 to 400 g of H2S/(m3bed·day) (curve 1). Therefore, it can be suggested that, under the current process operating conditions, the maximum elimination capacity corresponding to the maximum H2S removal efficiency is approximately 300 g of H2S/(m3bed·day). These results are also in agreement with the previous reported results (Soreanu et al. 2007).

Degradation Pathway

A previous macrokinetic study was undertaken in order to determine the H2S degradation pathway under constant H2S input and nitrate-limiting and non-limiting conditions (Soreanu et al. 2008). In the present study, the experiment was carried out under varied IL input and nitrate nonlimiting conditions (nitrate in excess with respect to the nitrate requirement). The excess of nitrate as was used in this study does not affect the H2S removal and assures a maximum potential of biodegradation over the entire period of the process (Soreanu et al. 2007, 2008). The formation of the corresponding degradation products was monitored as an indicator of hydrogen sulphide oxidation and the denitrification pathway.

The experimental variation of the nitrate degradation rate ($r_{\text{N-NO3}}$, mg of N-nitrate consumed/L/day), nitrite formation rate ($r_{\text{N-NO2}}$, mg of N-nitrite formed/L/day) and sulphate formation rate ($r_{\text{S-SO4^2-}}$, mg of S-sulphate formed/L/day) versus EC' (S-H2S removed/day) are presented in Fig. 5. As can be observed, the nitrate degradation rate increased with EC' from 10.31 mg/L/day to a maximum of 32.57 mg/L/day, after which it remained constant at approximately 30 mg/L/day. Nitrite and sulphate formation rates fluctuated at approximately $12 \pm 4$ mg/L/day and $9 \pm 2$ mg/L/day respectively, in the range of EC' tested, thus suggesting that nitrogen and elemental sulphur are preferentially produced when the IL/EC is increased.

Degradation and formation rates, as determined previously by Soreanu et al. (2008) for a loading of 1.5 g of H2S per day were: $r_{\text{N-NO3}} = 20.53$ mg of N-NO3/L/day; $r_{\text{N-NO2}} = 6.41$ mg of N-NO2/L/day; $r_{\text{SO4^2-}} = 9.09$. These rates are in agreement with the results obtained in this study for a similar loading (i.e., for 1.35 g of H2S per day, these rates are: $r_{\text{N-NO3}} = 18.99$ mg of N-NO3/L/day; $r_{\text{N-NO2}} = 9.02$ mg of N-NO2/L/day; $r_{\text{SO4^2-}} = 11.08$). Overall, it appears that the increase of H2S loading rate occurs with the increase of S0/SO42- and N2/NO2-, thus it dictates the ratio of degradation products in the system. The observed, moderately constant nitrate degradation and nitrite formation rates for IL' > 2.24 g of S-H2S feed per day [168 g of H2S feed/(m3bed-day)] or EC' > 2.11 g of S-H2S removed per day (Fig. 5), suggest that the ratio N2/NO2- is not significantly influenced by changes in S-input in the IL' tested range of 2.24 to 7.24 g of S-H2S feed per day corresponding to an EC' between 2.11 to 5.12 g of S-H2S removed per day.

Figures 6 and 7 present the ratio of S and N degradation products versus IL as obtained from the corresponding mass balance. According to Fig. 6, the amount of sulphate decreases from 50.46% down to 8.87% with an increase of IL from 24.04 to 168.32 g of H2S/(m3bed-day), and further decreases to trace levels at higher IL. This behaviour suggests that sulphate may be the major S-degradation
product at low IL [i.e., <24.04 g of H₂S/(m³bed·day)] and elemental sulphur becomes the major S-degradation product at higher IL [i.e. >96.18 g of H₂S/(m³bed·day)]. For example, for an IL of 96.18 g of H₂S/(m³bed·day) (or 1.36 g of H₂S feed per day and, respectively, 1.27 g of S-H₂S removed per day), H₂S degradation occurs with the formation of 20.40% S-SO₄²⁻ and 79.59% S as Sₒ. The formation of elemental sulphur as the major degradation product seems to be related to a high IL, as has been reported by other authors (Buisman et al. 1990; Chung et al. 1996; Gevertz et al. 2000; McComas and Sublette 2001; Soreanu et al. 2007) either under aerobic or anoxic conditions. For example, under anoxic conditions and at a loading of 1.5 g of H₂S per day, Soreanu et al. (2008) reported the formation of approximately 15.25% S-sulphate and 84.75% elemental sulphur, which is in agreement with the above-mentioned results for similar loadings.

According to Fig. 7, the amount of nitrite produced decreases from 99.9 to 21.46% when the IL increases from 24.04 to 168.32 g of H₂S/(m³bed·day), and fluctuates between 53.46 and 29.89% at higher IL. This observation suggests that at low available H₂S, nitrate is preferentially used in the nitrate-nitrite competition for the substrate (H₂S) (Tiedje 1988). Otherwise, at higher IL [i.e., >96.18 g of H₂S/(m³bed·day)], both nitrate and the reduced nitrite are consumed with the formation of nitrogen, usually as the main degradation product. Hence, the nitrate degradation pathway was dependent on the H₂S loads tested in this study, especially at smaller loading rates. Nitrate degradation during IL = 96.18 g of H₂S/(m³bed·day) (or 1.35 g of H₂S per day) occurs with the formation of 47.50% N-nitrite and 52.50% N as N₂; that is comparable to results obtained by Soreanu et al. (2008) for a similar H₂S charge (1.5 g of H₂S per day) (i.e., 31.22% N-nitrite and 68.78% N as N₂). Gevertz et al. (2000) observed nitrate reduction at different sulphide (S²⁻) concentrations using two novel chemolithotrophic nitrate-reducing, sulphide-oxidizing bacterial strains that were isolated from oil field brine and identified as *Thiomicrrocysta denitrificans* (CVO) and genus *Arcobacter* (FWKO B). For CVO, a similar behaviour was obtained where the nitrite was observed to be the dominant N-degradation product (>60%) at low S²⁻ concentrations, compared with <50% at higher S²⁻ concentrations (2 and 3 mM, or 64 and 96 mg of S²⁻ per L). For FWKO B, nitrate reduction occurred with only nitrite as a degradation product, independent of S²⁻ concentration.

Interestingly, the stabilization of sulphate and nitrite formation is observed at the same IL [≥168.32 g of H₂S/(m³bed·day)] (Fig. 6 and 7), suggesting that a different degradation pathway at high H₂S loading rates is followed (i.e., with the formation of mainly elemental sulphur and nitrogen). This observation is in agreement with McComas and Sublette (2001) who report that elemental sulphur becomes the major product of H₂S degradation involving *Thiobacillus denitrificans* when the H₂S loading rates exceed the maximum oxidation rate (i.e. when RE begins to decrease). Indeed, the change in the ratio of the degradation products is the result of the change of the degradation mechanism due to the change in the biological activity of the microorganisms with the load of the substrate in the system.

It appears that under non nitrate-limiting conditions, the degradation pathway of hydrogen sulphide was independent of the nitrate degradation pathway. Similarly, Soreanu et al. (2008) observed that the hydrogen sulphide degradation pathway at constant S input was not significantly influenced by the change in the N to S ratio resulting from changes in N input under non nitrate-limiting conditions. This behaviour is possible, taking into consideration that if one of the compounds (i.e., nitrate) is in excess, then the ratio of the degradation products (Sₒ/SO₄²⁻, and, respectively, N₂/NO₂⁻) depends on the initial concentration of the limited reactant, i.e., H₂S.

The modelling of the dual biological process for both sulphide and nitrate under limiting and nonlimiting conditions would be beneficial in order to predict the degradation pathway as a function of S or N changes occurring in the system.

Therefore, the reaction schemes (equations 1 to 10) shown in Table 1 can be drawn in order to describe the sulphur and nitrogen balance observed and thus the degradation pathway as a function of IL (note: trace
constituents have not been considered). Moreover, the linear variation of sulphate and nitrite percentages observed for IL ranging between 24.05 and 168.32 g of H₂S/(m³bed·day) allows the prediction of the degradation product ratio as a function of IL from the corresponding linear regressions: (Percentage Compound, %) = f(IL·RE) (equations 11 to 14).

![Image](Image315x96 to 547x251)

<table>
<thead>
<tr>
<th>IL *</th>
<th>Reaction scheme</th>
<th>Equation No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.05</td>
<td>S-H₂S → 0.51 S-SO₄²⁻ + 0.49 S⁰</td>
<td>(1)</td>
</tr>
<tr>
<td></td>
<td>N-NO₃⁻ → N-NO₂⁻</td>
<td>(2)</td>
</tr>
<tr>
<td>96.18</td>
<td>S-H₂S → 0.20 S-SO₄²⁻ + 0.80 S⁰</td>
<td>(3)</td>
</tr>
<tr>
<td></td>
<td>N-NO₃⁻ → 0.47 N-NO₂⁻ + 0.53 N-N₂</td>
<td>(4)</td>
</tr>
<tr>
<td>168.32</td>
<td>S-H₂S → 0.09 S-SO₄²⁻ + 0.91 S⁰</td>
<td>(5)</td>
</tr>
<tr>
<td></td>
<td>N-NO₂⁻ → 0.22 N-NO₃⁻ + 0.78 N-N₂</td>
<td>(6)</td>
</tr>
<tr>
<td>381.10</td>
<td>S-H₂S → S⁰</td>
<td>(7)</td>
</tr>
<tr>
<td></td>
<td>N-NO₂⁻ → 0.54 N-NO₃⁻ + 0.46 N-N₂</td>
<td>(8)</td>
</tr>
<tr>
<td>543.96</td>
<td>S-H₂S → S⁰</td>
<td>(9)</td>
</tr>
<tr>
<td></td>
<td>N-NO₂⁻ → 0.29 N-NO₃⁻ + 0.71 N-N₂</td>
<td>(10)</td>
</tr>
</tbody>
</table>

*IL units, g of H₂S/(m³bed·day)

Subject to experimental variation

S⁰, % = 0.311x + 44.512  
N₂, % = 0.577x - 8.8574  
N-Nitrite, % = -0.577x + 108.86  
S-Sulphate, % = -0.311x + 55.488

Where x = IL [g of H₂S/(m³bed·day)]·RE (%); these equations are suitable for IL = 24.04 to 168.32 g of H₂S/ (m³bed·day).

**Nitrate Demand**

Figure 8 shows the variation of nitrate demand (g of N-NO₃⁻ per g of H₂S removed) versus the amount of hydrogen sulphide removed per day (EC', g of H₂S removed per day). The process is characterized by chemical and biological composites that change as a result of reaction conditions. Experimentally, it was observed that nitrate demand (g of N-NO₃⁻ consumed per g of H₂S degraded) decreases from 0.71 to 0.12 g of N-NO₃⁻ per g of H₂S removed with an increase in the amount of H₂S degraded from 0.34 to 5.42 g of H₂S removed per day. This behaviour may be possible with regard to the experimentally determined degradation pathways presented in Table 1, and to the theoretical reactions and their corresponding theoretical nitrate demands previously presented in Soreanu et al. (2008). The degradation pathways presented in Table 1 were determined from the sulphur and nitrogen balances. As can be seen, at small H₂S loading rates [IL = 24.05 g of H₂S/(m³bed·day)] used in this study, H₂S degradation occurred with the formation of both sulphate and elemental sulphur, while nitrate degradation occurred mainly with the formation of nitrite. These reactions agree with the theoretical reactions (7) and (8) from Soreanu et al. (2008), corresponding to the highest theoretical nitrate demand (i.e., average of 1.02 g of N-NO₃⁻ consumed per g of H₂S degraded), as also observed in this study (i.e., 0.71 g of N-NO₃⁻ consumed per g of H₂S degraded). The smallest nitrate demand (i.e., 0.13 to 0.15 g of N-NO₃⁻ consumed per g of H₂S degraded) recorded in this study was observed at a high H₂S loading rate [i.e., IL ≥ 168.32 g of H₂S/(m³bed·day)] when hydrogen sulphide was degraded to mainly elemental sulphur, while nitrate degradation occurred with the formation of predominantly nitrogen, and the balance was nitrite. Indeed, according to Soreanu et al. (2008), the smallest theoretical nitrate demand (i.e., 0.16 g of N-NO₃⁻ consumed per g of H₂S degraded) may occur when elemental sulphur and nitrogen are the main degradation products. At the medium H₂S loading rates [i.e., 96.18 g of H₂S feed/(m³bed·day) or 1.36 g of H₂S feed per day] used in this study, elemental sulphur and sulphate were the major and minor products of biodegradation, respectively, while nitrite and nitrogen were also present in the system as a result of nitrate degradation. Nitrate demand recorded under such conditions was 0.33 g of N-NO₃⁻ consumed per g of H₂S degraded. A similar ratio of degradation products and nitrate demand was experimentally and theoretically obtained by Soreanu et al. (2008) for a similar H₂S loading rate. As can be seen in Fig. 8, the nitrate demand remained constant at 0.33 ± 0.01 g of N-NO₃⁻ consumed per g of H₂S degraded for EC' between 1.01 and 2.24 g of H₂S removed per day.

**Operation with Two Bioreactors in Series**

In practice, a prefilter may be used in order to reduce the loading of pollutants to the second filter and thus increase the overall performance of the biofiltration system. This dual bioreactor design was thus undertaken using two bioreactors in series (i.e., 0.012 and 0.014 m³, respectively). As can be seen in Fig. 9, the RE increased from 35.14% after one reactor [when 793.33 g of H₂S/(m³bed·day)] was fed in the 0.012 m³ reactor] to a
cumulative RE of 90.6% after two reactors in series. In another experimental trial, the RE increased from 52.73% [when 537 g of H2S/(m3bed·day) was fed in the 0.012 m3 reactor] to a cumulative RE of 98.02% after two reactors in series. These results suggest that under the above-mentioned conditions, the RE can be improved up to 2.5 times with the addition of a second reactor. As expected, the addition of the second reactor decreased the overall IL by increasing the total packing volume to 0.026 m3, which resulted in an EC quite close to the EC observed when a similar IL was loaded in one reactor (0.014-m3 bed volume). These results indicate that the packing media volume and/or the type of packing material is another significant factor to be considered for the improvement of the process performance, especially for higher H2S loading rate applications.

Regeneration and Sulphur Recovery from Packing Media

One of the major problems reported for biofilters is the excessive production of the biomass leading to the fouling of packing media and the increase of the pressure drop across the biofilter (Devinn et al. 1999). These problems were not observed in present and previous similar studies, likely due to the anoxic characteristic of the process (Soreanu et al. 2008). Elemental sulphur production, although visually observed as a very fine light yellow powder, does not interfere with the operation of the biofilter (i.e., clogging), probably due to its hydrophilic nature (Seidel et al. 2006). A very simple maintenance procedure to remove excess sulphur is recommended, and involves the occasional flushing of the column with nutrient solution (i.e., monthly, depending on process). This procedure was carried out in order to avoid excess accumulation of elemental sulphur in the packing media.

This procedure was also successfully applied in previous studies, without any negative effect on the operational and process performance of the biofilter. The recovery of sulphur from the “washing” solution is possible, if desired. Sulphur recovery was not a main purpose of this study, but it is interesting to note that recovery of the sulphur deposited on the fibres was also possible by drying and shaking the plastic fibre media. Table 2 presents a summary characterization of the sulphur recovered from the plastic fibres. High sulphur purity was obtained (83.25% sulphur), thus offering new technology perspectives.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>% (per solid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphur (extractable)</td>
<td>83.25</td>
</tr>
<tr>
<td>Sulphate</td>
<td>1.04</td>
</tr>
<tr>
<td>Thiosulphate</td>
<td>ND</td>
</tr>
<tr>
<td>Iron (extractable)</td>
<td>0.628</td>
</tr>
<tr>
<td>Sodium (extractable)</td>
<td>2.59</td>
</tr>
<tr>
<td>Ammonia as N</td>
<td>ND</td>
</tr>
<tr>
<td>Nitrite as N</td>
<td>0.27</td>
</tr>
<tr>
<td>Nitrate as N</td>
<td>1.06</td>
</tr>
<tr>
<td>Other impurities * / losses</td>
<td>11.16</td>
</tr>
</tbody>
</table>

*ND = not detected.
*Other impurities, particles of biomass, fibres, etc.

Conclusion

This paper presents a study on the performance of an anoxic biotrickling filter treating H2S in biogas. The experiments were carried out at several loading rates ranging between IL = 20 to 550 g of H2S/(m3bed·day) under nitrate-nonlimiting conditions. The maximum elimination capacity corresponding to the maximum H2S removal efficiency (>95%) was found to be approximately 300 g of H2S/(m3bed·day). An increase of the IL to a level more than this value resulted in a decrease of RE. The operation of two bioreactors in series demonstrated a significant improvement in process performance. The H2S degradation pathway was also found to be influenced by the change in IL. At small to medium IL [i.e., 24.05 to 96.18 g of H2S/(m3bed·day)], the H2S degradation occurs with the formation of both sulphate and elemental sulphur, while at higher IL [IL ≥ 168.32 g of H2S/(m3bed·day)], elemental sulphur is the major degradation product. It also appears that under non nitrate-limiting conditions, the degradation pathways of the hydrogen sulphide were independent of the nitrate degradation pathway, while the nitrate degradation pathway was dependent on the H2S loading rate, when, for example, nitrogen formation was favoured at high H2S loading rates [IL ≥ 168.32 g of H2S/(m3bed·day)]. The technology presented requires simple maintenance of the biotrickling filter, and sulphur recovery is also possible.
Acknowledgments

The authors wish to thank Natural Resources Canada's Decentralized Energy Production Initiative, Environment Canada, and the Natural Sciences and Engineering Research Council of Canada for funding and administration of this research. They also wish to thank John Salvatore and Mohamad Al-Jamal (Water Science & Technology Directorate, Environment Canada) for their important contributions to this study.

References


Étude comparative de deux flocculants pour le traitement physico-chimique d’une eau usée municipale : chitosane et polymère de synthèse

Comparative Study of Two Flocculants in the Physical-Chemical Treatment of Municipal Wastewater: Chitosan and a Synthetic Polymer

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2Chargé de projets, CIMA+, 740, rue Notre-Dame ouest, Montréal (QC) H3C 3X6
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Le traitement des eaux usées municipales par voie physico-chimique requiert généralement l’utilisation d’un coagulant à base de sels métalliques et d’un flocculant (ou aide-coagulant) synthétique. L’application du concept de développement durable dans le traitement de ces eaux privilégie une approche utilisant des produits renouvelables tels les biopolymères naturels. Des essais avec du chitosane (biopolymère naturel) comme flocculant ont été effectués à l’échelle du laboratoire (jar test) et à l’échelle réelle (station municipale de type physico-chimique) afin de mieux comprendre les particularités liées à l’utilisation de ce produit naturel lors du traitement des eaux. Les essais à l’échelle réelle réalisés dans deux filières parallèles de traitement ont comparé l’utilisation de la combinaison alun et polymère de synthèse anionique (AL/PS) à la combinaison alun et chitosane (AL/CH). Des abattements de la DCO de 87 %, des MES de 95 % et de 93 % pour le P tot ont été observés avec la combinaison AL/CH. Ces résultats d’abattement de la DCO et des MES sont semblables à ceux obtenus dans la filière de traitement AL/PS. Certains essais indiquent un dosage de coagulant (alun) inférieur de 24,8 % avec la combinaison AL/CH. L’abattement du P tot est constamment plus élevé avec la combinaison AL/PS en raison du dosage plus élevé de coagulant.

Mots clés : Alun, chitosane, coagulant, eau usée municipale, flocculant, polymère

The treatment of municipal wastewaters by physical-chemical methods normally requires the use of a metallic salt coagulant and a synthetic coagulant aid. Integrating the sustainable development concept in the treatment of waters favours the use of renewable resources such as natural biopolymers. In order to better understand the peculiarities of using a product of natural origin in municipal wastewater treatment, laboratory testing (jar tests) was achieved with chitosan as a coagulant aid, as well as full-scale testing in a medium size physical-chemical wastewater treatment plant. The full-scale test was performed in two parallel, identical systems treating the same wastewater under the same conditions. The one using a combination of alum with a synthetic polymer (AL/SP) was compared with the other which used alum and chitosan (AL/CH). Removals for COD, SS, and total phosphorus reached 87%, 95% and 93%, respectively, for the AL/CH combination. These results are similar to those obtained for COD and SS with the AL/SP combination. Some results show a coagulant dosage (alum) up to 24.8% lower with chitosan as the usual coagulant aid. For total phosphorus, however, the results show that removals were higher with the AL/SP combination because of a higher coagulant dosage.

Key words: Alum, chitosan, coagulant, flocculant, municipal wastewater, polymer

Introduction

Les installations d’assainissement des eaux usées de type physico-chimique utilisent aujourd’hui des procédés ayant des charges hydrauliques élevées et des temps de rétention relativement courts. La pratique dans ce domaine d’application permet la mise en place d’unités de décantation dont les charges hydrauliques superficielles peuvent atteindre 15 à 80 m/h selon le fabricant et les conditions. L’atteinte des objectifs de traitement, soit l’abattement des matières en suspension (MES), du phosphore total (P tot), de l’azote total (N tot) et des indicateurs microbiologiques, requiert généralement l’utilisation de coagulant à base de sels métalliques. Les sels d’aluminium et les sels ferriques sont les coagulants les plus couramment utilisés en traitement physico-chimique des eaux (Viessman et Hammer 1993; Kawamura 1991a;
Degremont 1989). L'utilisation de ces sels métalliques implique l'usage de floculants (ou aide-coagulants) de synthèse, généralement des polyacrylamides de synthèse cationiques, anioniques ou non-ioniques.

Bien que l'efficacité des produits de synthèse soit largement documentée, des difficultés (surdosage, perte d'abattement, apparition d'odeurs) sont observées avec ce type de produits sous certaines conditions de traitement. De plus, l'ajout de ces produits chimiques dans les procédés génère des boues contenant des substances limitant ou empêchant leur valorisation (Polan et Jones 1992; Kawamura 1991b). D'un point de vue environnemental, l'utilisation de ces produits chimiques ne respecte pas nécessairement certains des principes du développement durable.


D'autres limitations potentielles peuvent se présenter; les formulations polymériques peuvent contenir des impuretés comme des monomères résiduels, des sous-produits de réaction et d'autres réactifs qui pourraient avoir un impact négatif sur la santé humaine. Les polymères et les substances indésirables qui leur sont associées peuvent réagir avec les produits dans l'eau ou ceux ajoutés lors du traitement et créer des sous-produits dont les effets sur la santé sont inconnus (Ozacar et Sengil 2003). Il est recommandé (Aguilar et al. 2005) d'examiner et de prendre en considération les propriétés toxiques de chaque polymère synthétique considéré pour ajout à l'eau. D'où la nécessité de poursuivre la recherche de solutions alternatives utilisant des produits ou matières répondant mieux aux principes du développement durable. Dans cet article, le chitosane est considéré comme floculant alternatif.


Cet article rapporte et discute les résultats d'essais réalisés à une station de traitement des eaux usées de type physico-chimique d'une municipalité de taille moyenne ne comportant pas d'industries, localisée au nord de Montréal (Québec, Canada).

**Méthodologie**

**Objectifs des essais**

Des essais ont été réalisés aux échelles du laboratoire et réelle. Deux coagulants sont utilisés à l'échelle du laboratoire, soit le sulfate d'aluminium ou alun (AL) et le sulfate ferrique (SF), et un seul à l'échelle réelle : l'alun. Deux floculants sont employés à chaque échelle : un copolymère d'acrylamide anionique, le Prosédim AS-32 (PS) et un biopolymère cationique, le chitosane (CH).

Le principal objectif de ces essais consiste à comparer le rendement des deux combinaisons de coagulant et de floculant.

Dans cette station de traitement des eaux usées, une combinaison de coagulant et floculant est déjà utilisée et éprouvée depuis plusieurs années : l'alun et le Prosédim AS-32 (AL/PS). Dans le cadre de ces essais comparatifs, l'autre combinaison de coagulant et floculant employée est l'alun et le chitosane (AL/CH).

**Essais à l'échelle du laboratoire (jar test)**

**Échantillonnage et conditions d'essais.** Dans une première étape, des essais à l'échelle du laboratoire (jar test) sont réalisés en condition d'hiver (avant la fonte de la neige et l'infiltration des eaux). L'objectif de ces essais est double : 1) valider la compatibilité entre les coagulants et floculants, 2) comparer les dosages obtenus avec ceux normalement utilisés dans le procédé de la station. Le tableau 1 présente les caractéristiques des équipements utilisés ainsi que les conditions dans lesquelles ces essais sont menés.

Un échantillon ponctuel de 70 litres d'eau usée, prélevé dans le canal d'affluent de la station pour effectuer ces essais, est conservé dans un réservoir cylindrique en
Comparaison de flocsulants – traitement d’eau usée

TABLEAU 1. Description des équipements et des conditions d’essais

<table>
<thead>
<tr>
<th>Matériel</th>
<th>Description</th>
</tr>
</thead>
</table>
| Bécher (réacteur de mélange) | Marque : Phipps & Bird Inc.  
Modèle : B-KER³. Bécher carré de 2 litres en plexiglass avec point d’échantillonnage intégré localisé dans la partie inférieure au tiers de la hauteur. |
| Banc d’essais (mélangeur) | Marque : Phipps & Bird Inc.  
Modèle : Stirrer 7790-400. Cadran digital, vitesse 0 – 330 tpm, 2 vitesses variables. |
| Matériel de dosage        | Seringues 0 – 1 mL, 0 – 5 mL.                                                |

<table>
<thead>
<tr>
<th>Condition de mélange</th>
<th>Débit du système d’échantillonnage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temps (min)</td>
<td>Rapide</td>
</tr>
<tr>
<td>Temps (min)</td>
<td>1</td>
</tr>
<tr>
<td>Vitesse (tpm)</td>
<td>100</td>
</tr>
</tbody>
</table>

TABLEAU 2. Caractéristiques des produits chimiques et naturel utilisés

<table>
<thead>
<tr>
<th>Produit</th>
<th>Caractéristiques</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>COAGULANT</strong></td>
<td></td>
</tr>
</tbody>
</table>
| Sulfate d’aluminium (Al) | Type de produit : Sel métallique (Al₂(SO₄)₃ en solution)  
Aspect : Liquide  
Concentration du produit : 48,8 %  
Concentration de Al₂O₃ : 8,25 % |
| Sulfate ferrique (SF)    | Type de produit : Sel métallique (Fe₂(SO₄)₃ en solution)  
Aspect : Liquide  
Concentration du produit : 43 à 50 %  
Concentration de Fe : 12 ± 0,5 % |
| **FLOCULANT**            |                                                      |
| Prosédim AS-32 (PS)      | Type de produit : Copolymère d’acrylamide  
Aspect : Poudre  
Viscosité Brookfields : 1600 cps (20°C et à 5 g/L)  
Ionicité : Anionique |
| Chitosane Kitomer (CH)   | Type de produit : Biopolymer naturel  
Aspect : Flocon  
Viscosité Brookfields : 1 000 à 2 500 cps (1,0%)  
Degré de déacétylation : 81 %  
Poids moléculaire : 2 000 000 dalton  
Ionicité : Cationique |

TABLEAU 3. Produits et plages de dosage utilisés pour les jar tests

<table>
<thead>
<tr>
<th>Coagulant</th>
<th>Plage de dosage (mg/L)</th>
<th>Floculant</th>
<th>Plage de dosage (mg/L)</th>
<th>Combinaison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfate d’aluminium</td>
<td>45 – 70</td>
<td>Prosédim AS-32</td>
<td>0,3</td>
<td>AL/PS</td>
</tr>
<tr>
<td></td>
<td>30 – 70</td>
<td>Chitosane</td>
<td>0,1 – 0,3</td>
<td>AL/CH</td>
</tr>
<tr>
<td>Sulfate ferrique</td>
<td>50 – 70</td>
<td>Prosédim AS-32</td>
<td>0,3</td>
<td>SF/PS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chitosane</td>
<td>0,3</td>
<td>SF/CH</td>
</tr>
</tbody>
</table>

plastique. Les essais jar test sont réalisés dans les heures qui suivent. Le moment du prélèvement correspond à une période de débit et de charge élevés dans la journée. 

Produits chimiques et dosages. Les caractéristiques des produits chimiques et naturel ainsi que leurs dosages respectifs sont présentés aux tableaux 2 et 3.
Essais à l'échelle réelle

La seconde étape consiste à réaliser des essais comparatifs à l'échelle réelle dans la station. Ces conditions d'essais comparatifs sont rendues possibles en raison de l'existence de deux filières identiques de traitement en parallèle à la station. Cette caractéristique permet la mise en place de stratégies de traitement différentes dans chacune des filières de coagulation – flocculation – décantation fonctionnant en parallèle. Cette configuration a rendu possible la comparaison simultanée de deux combinaisons de traitement (coagulant / flocaulant) sur des eaux usées de mêmes caractéristiques physico-chimiques soumis aux mêmes conditions hydrauliques.

Caractéristiques de la station et objectifs de rejet. Les eaux usées brutes sont relevées dans la station par deux postes permettant une modulation du pompage sans régularisation de débit. À leur entrée dans la station, les eaux usées sont dirigées vers les dégrilleurs puis le débit est mesuré à l'aide d'un canal Parshall. Les eaux s'écoulent ensuite vers des dessableurs très compacts munis d'aérateurs à grosses bulles. Les eaux dessablées sont dirigées vers les unités de mélange rapide et lent dans lesquels les produits chimiques sont ajoutés. Les graisses et les écumes sont recueillies à la surface des unités de flocculation munies de ponts roulants. Elles sont ensuite dirigées dans les digesteurs anaérobies avec les boues physico-chimiques décantées.

Dans chaque filière, l'eau conditionnée par un coagulant métallique et un flocaulant subit les étapes de coagulation – flocculation – décantation. Les ouvrages permettant ce processus sont successivement le mélangeur rapide, le mélangeur lent et le décanteur. Les unités de mélange rapide et lent consistent en des bassins de forme rectangulaire munis de mélangeur à hélice et qui ont respectivement des puissances de 3,7 et 5,6 kW. Au débit de conception de 21 343 m$^3$/d, les temps de résidence moyens des deux mélangeurs rapide et lent, sont respectivement de 4,7 et 15,8 minutes. Les points de dosage du coagulant et flocaulant sont respectivement situés à l'entrée du mélangeur rapide et du mélangeur lent.

Les décanteurs, du type à compartiments en alvéole inclinés, sont conçus pour rencontrer des charges hydrauliques surfaciques pouvant atteindre 30 m/h. Au débit de conception, le temps de résidence moyen est de 77,5 minutes avec une vitesse ascensionnelle moyenne de 8,4 m/h. Ces décanteurs permettent la recirculation des boues, mais ce dispositif n’a pas été utilisé lors des essais. L’extraction des boues des décanteurs vers les digesteurs anaérobies s’effectue en mode discontinu selon une séquence permettant le maintien du niveau du voile de boues dans les conditions d’usage d’opération.

Les eaux décantées subissent finalement une désinfection par rayonnement ultra-violet avec des lampes de type basse pression à haute intensité. Le canal d’évacuation de la station achemine les eaux dans la rivière des Mille-Îles par un émissaire submergé.

Les débits de conception et les objectifs de rejet de la station sont respectivement présentés aux tableaux 4 et 5.


<table>
<thead>
<tr>
<th>Paramètre</th>
<th>Période</th>
<th>Concentration à l’effluent (mg/L) ou enlèvement</th>
<th>Charge allouée à l’effluent (kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MES</td>
<td>Annuelle</td>
<td>20 (ou 75 %)</td>
<td>579</td>
</tr>
<tr>
<td></td>
<td>Mensuelle</td>
<td>30 (ou 65 %)</td>
<td>798</td>
</tr>
<tr>
<td>$P_{tot}$</td>
<td>Annuelle</td>
<td>0,5 (ou 70 %)</td>
<td>19,5</td>
</tr>
<tr>
<td></td>
<td>Mensuelle</td>
<td>0,75 (ou 60 %)</td>
<td>26</td>
</tr>
<tr>
<td>Coliformes fécaux</td>
<td>1er juin au 30 septembre</td>
<td>500 ufc / 100 mL</td>
<td>Moyenne géométrique avant réactivation</td>
</tr>
<tr>
<td></td>
<td>1er octobre au 31 mai</td>
<td>4 000 ufc / 100 mL</td>
<td>Moyenne géométrique avant réactivation</td>
</tr>
</tbody>
</table>

*Source : Babineau et al. 1999a, 1999b.
Comparaison de floculants – traitement d’eau usée

Lors des essais, des échantillons sont prélevés à l’aide de trois (3) échantillonneurs automatiques proportionnels au débit (composés sur 24 heures). Un premier échantillonneur est localisé à l’affluent de la station; les deux autres à la sortie des décanteurs.

Analyses de laboratoire
Les analyses des paramètres physico-chimiques sont réalisées rapidement avec les appareils présents sur le site. Les paramètres retenus et les méthodes d’analyses utilisées sont indiqués au tableau 7.

Échantillonnage et contrôle de la qualité. Lors des essais, des échantillons sont prélevés à l’aide de trois (3) échantillonneurs automatiques proportionnels au débit (composés sur 24 heures). Un premier échantillonneur est localisé à l’affluent de la station; les deux autres à la sortie des décanteurs.

Analyses de laboratoire
Les analyses des paramètres physico-chimiques sont réalisées rapidement avec les appareils présents sur le site. Les paramètres retenus et les méthodes d’analyses utilisées sont indiqués au tableau 7.

Résultats

Essais à l’échelle du laboratoire

Les résultats des essais réalisés à l’échelle du laboratoire sont présentés au tableau 8. Ils sont regroupés en trois séries (A, B et C) réalisées sur le même échantillon d’eau usée. Ces trois séries d’essais permettent de comparer quatre différentes combinaisons de coagulant et floculant à différents dosages. Les dosages retenus pour ces essais permettent d’explorer une plage de dosage comparable avec celle couramment utilisée en usine. De plus, la plage de dosage examinée dans cette partie des essais permet d’établir une première base de résultats pour les essais à l’échelle réelle. La figure 1 montre les abattements de DCO, Ptot, Tu (turbidité) et MES pour les 4 combinaisons au dosage de 70 mg/L de coagulant et 0,30 mg/L de floculant.

Essais à l’échelle réelle

Variation du débit. Les débits instantanés enregistrés à l’affluent de la station d’épuration lors de la période des essais (juillet et août) ont variés de 4 630 m³/d à 37 750 m³/d. Le débit moyen journalier pendant cette même période était de 17 900 m³/d avec un coefficient de variation de 4 %.

Les fluctuations horaires du débit observées quotidiennement correspondent à un cycle journalier typique lié aux activités d’une municipalité de taille moyenne constituée exclusivement de secteurs résidentiels et commerciaux.

Caractérisation de l’affluent. La figure 2 présente une caractérisation des eaux usées de l’affluent pour la période des essais à l’échelle réelle. Les paramètres retenus sont la DCO, les MES et le Ptot. Les valeurs de ces paramètres sont typiques des concentrations observées sur une base annuelle à la station, tel qu’indiqué au tableau 6.

Comparaisons d’abattement. Les dosages moyens d’alun et de floculant utilisés dans chacun des décanteurs pour la période des essais sont présentés à la figure 3. Les figures 4 à 8 exposent respectivement la variation du pH et de l’alcalinité de même que les abattements du Ptot, des MES et de la DCO dans l’effluent des décanteurs pour chacune des deux filières parallèles.

### Tableau 6. Produits et plages de dosage utilisés à l’échelle réelle

<table>
<thead>
<tr>
<th>Filière no. 1</th>
<th>Plage de dosage (mg/L)</th>
<th>Filière no. 2</th>
<th>Plage de dosage (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Produits</td>
<td></td>
<td>Produits</td>
<td></td>
</tr>
<tr>
<td>Coagulant</td>
<td>Sulfate d’aluminium</td>
<td>43 - 54</td>
<td>Sulfate d’aluminium</td>
</tr>
<tr>
<td>Floculant</td>
<td>Prosédim AS-32</td>
<td>0,30 - 0,35</td>
<td>Chitosane</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0,08 - 0,44</td>
</tr>
</tbody>
</table>

### Tableau 7. Paramètres physico-chimiques et méthodes d’analyse

<table>
<thead>
<tr>
<th>Paramètres</th>
<th>Méthodes d’analysea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance UV à 254 nm</td>
<td>Méthode d’absorption ultraviolet no. 5910 B</td>
</tr>
<tr>
<td>Alcalinité</td>
<td>Méthode par titration no. 2320 B</td>
</tr>
<tr>
<td>Demande chimique en oxygène (DCO)</td>
<td>Méthode par digestion no. 5220 D</td>
</tr>
<tr>
<td>Fer</td>
<td>Méthode à la phénantroline no. 3500-Fe B</td>
</tr>
<tr>
<td>Matière en suspension (MES)</td>
<td>Méthode par filtration et séchage (103 -105 °C) no. 2540 D</td>
</tr>
<tr>
<td>pH</td>
<td>Méthode électrométrique no. 4500-H³ B</td>
</tr>
<tr>
<td>Phosphore total (Ptot)</td>
<td>Méthode par digestion au persulfate en milieu acide no. 4500 H</td>
</tr>
<tr>
<td>Turbidité (Tu)</td>
<td>Méthode néphéломétrique no. 2130 B</td>
</tr>
</tbody>
</table>

aL’analyse fait référence aux méthodes APHA et al (1999)
### Tableau 8. Résultats des essais à l'échelle du laboratoire

<table>
<thead>
<tr>
<th>Essais</th>
<th>Coagulant</th>
<th>Floculant</th>
<th>Absorbance</th>
<th>pH</th>
<th>DCO</th>
<th>Ptot</th>
<th>Tu</th>
<th>Fer</th>
<th>MES</th>
<th>Observations visuelles du floc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AL mg/L</td>
<td>SF mg/L</td>
<td>PS mg/L</td>
<td>CH mg/L</td>
<td>UV à 254 nm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A*</td>
<td>70</td>
<td>0,3</td>
<td>—</td>
<td>—</td>
<td>0,38</td>
<td>6,9</td>
<td>121</td>
<td>1,1</td>
<td>12,2</td>
<td>0,07</td>
</tr>
<tr>
<td>2A</td>
<td>70</td>
<td>—</td>
<td>—</td>
<td>0,3</td>
<td>0,30</td>
<td>7,0</td>
<td>100</td>
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<td>5,2</td>
<td>0,03</td>
</tr>
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<td>4A</td>
<td>50</td>
<td>—</td>
<td>—</td>
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<td>0,39</td>
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<td>40</td>
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<tr>
<td>6A</td>
<td>30</td>
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<td>0,69</td>
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**Série B**

<table>
<thead>
<tr>
<th>Essais</th>
<th>Coagulant</th>
<th>Floculant</th>
<th>Absorbance</th>
<th>pH</th>
<th>DCO</th>
<th>Ptot</th>
<th>Tu</th>
<th>Fer</th>
<th>MES</th>
<th>Observations visuelles du floc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AL mg/L</td>
<td>SF mg/L</td>
<td>PS mg/L</td>
<td>CH mg/L</td>
<td>UV à 254 nm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1B*</td>
<td>70</td>
<td>0,3</td>
<td>—</td>
<td>—</td>
<td>0,35</td>
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<td>8,4</td>
<td>0,06</td>
</tr>
<tr>
<td>2B</td>
<td>60</td>
<td>—</td>
<td>—</td>
<td>0,3</td>
<td>0,36</td>
<td>7,2</td>
<td>125</td>
<td>1,2</td>
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<td>0,05</td>
</tr>
<tr>
<td>3B</td>
<td>45</td>
<td>—</td>
<td>—</td>
<td>0,3</td>
<td>0,43</td>
<td>7,3</td>
<td>115</td>
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</tr>
<tr>
<td>4B</td>
<td>50</td>
<td>—</td>
<td>—</td>
<td>0,2</td>
<td>0,45</td>
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<td>132</td>
<td>3,2</td>
<td>15,9</td>
<td>0,09</td>
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<tr>
<td>5B</td>
<td>45</td>
<td>—</td>
<td>—</td>
<td>0,2</td>
<td>0,56</td>
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<td>143</td>
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</tr>
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<td>6B</td>
<td>50</td>
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<td>—</td>
<td>0,1</td>
<td>0,46</td>
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<td>132</td>
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<td>16,8</td>
<td>0,07</td>
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</tbody>
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**Série C**

<table>
<thead>
<tr>
<th>Essais</th>
<th>Coagulant</th>
<th>Floculant</th>
<th>Absorbance</th>
<th>pH</th>
<th>DCO</th>
<th>Ptot</th>
<th>Tu</th>
<th>Fer</th>
<th>MES</th>
<th>Observations visuelles du floc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AL mg/L</td>
<td>SF mg/L</td>
<td>PS mg/L</td>
<td>CH mg/L</td>
<td>UV à 254 nm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1C*</td>
<td>70</td>
<td>0,3</td>
<td>—</td>
<td>—</td>
<td>0,36</td>
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<tr>
<td>2C</td>
<td>70</td>
<td>—</td>
<td>—</td>
<td>0,3</td>
<td>0,30</td>
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<td>0,9</td>
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<td>7,0</td>
<td>137</td>
<td>1,1</td>
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<td>3,46</td>
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<td>—</td>
<td>—</td>
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<td>7,1</td>
<td>119</td>
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</table>

* Combinaison de produits chimiques et dosages utilisés à la station au moment de l'échantillonnage.
Figure 1. Abattement de la DCO, du P$_{tot}$, de la Tu et des MES pour 4 combinaisons à l'échelle du laboratoire.

Figure 2. DCO, MES et P$_{tot}$ dans l'affluent de la station.

Figure 3. Dosage en coagulant et floculant dans les décanteurs.

Figure 4. Comparaison du pH dans l'effluent des décanteurs.
Figure 5. Comparaison de l'alcalinité dans l'effluent des décanteurs.

Figure 6. Comparaison de l'abattement du Ptot dans l'effluent des décanteurs.

Figure 7. Comparaison de l'abattement des MES dans l'effluent des décanteurs.

Figure 8. Comparaison de l'abattement de la DCO dans l'effluent des décanteurs.
Discussion

Essais à l’échelle du laboratoire

L’analyse des résultats des essais (tableau 8) indique l’existence de certaines particularités associées au coagulant. La combinaison AL/PS, aux dosages respectifs de 70 mg/L et 0,3 mg/L, correspond à la condition de dosage à la station au moment de l’échantillonnage; cette combinaison permet d’établir un point de référence afin d’évaluer la performance des différentes combinaisons et dosages étudiés. À ces dosages, la combinaison AL/CH est celle qui présente les meilleurs abattements pour les MES, la turbidité, le Ptot et le fer (tableau 8 et figure 1). Les observations visuelles du floc appuient ces résultats.

La combinaison SF/CH, à ces mêmes dosages, démontre aussi le potentiel de celle-ci pour l’abattement de la turbidité, du fer, de la DCO et des MES. À ces dosages, la combinaison AL/CH est celle qui présente les meilleurs abattements pour les MES, la turbidité, le Ptot et le fer (tableau 8 et figure 1). Les observations visuelles du floc appuient ces résultats.

La combinaison SF/CH, à ces mêmes dosages, démontre aussi le potentiel de celle-ci pour l’abattement des MES et du Ptot. Cette combinaison est celle qui démontre aussi le potentiel de celle-ci pour l’abattement de la turbidité, du fer, de la DCO et des MES. À ces dosages, la combinaison AL/CH est celle qui présente les meilleurs abattements pour les MES, la turbidité, le Ptot et le fer (tableau 8 et figure 1). Les observations visuelles du floc appuient ces résultats.

Optimisation et dosages. Un des objectifs poursuivis consiste à explorer des plages de dosages du coagulant métallique (AL) et floculant naturel (CH). À cet égard, les plages de dosage de produits explorées dans la filière no. 1 (AL/CH) utilisant le floculant naturel ne font pas l’objet d’une optimisation comparativement aux plages de dosages de produits de la filière no. 2 (AL/PS) utilisant le floculant de synthèse. Les plages de dosage de cette dernière filière (AL/PS) correspondent à des dosages optimisés depuis plusieurs années dans une station conçue pour leur utilisation. Cette particularité se doit d’être considérée dans l’analyse des rendements obtenus puisqu’elle compare les résultats de la qualité de l’eau traitée expérimentant un nouveau type de floculant (chitosane) avec ceux d’un type de floculant connu et déjà éprouvé pour ce type d’installation.

Dosage, pH et abattement. À un dosage moindre d’alun dans la filière no. 1 (AL/CH), on observe que les valeurs du pH et de l’alcalinité sont légèrement inférieures à celles observées dans la filière no. 2 (AL/PS). Les valeurs plus faibles de pH et d’alcalinité observées sont attribuables à l’acide utilisé pour la solubilisation du chitosane. Outre certaines de ces valeurs obtenues vers la fin de la troisième phase d’essais (8 au 12 août), aucun effet significatif sur l’alcalinité et le pH n’est observé. On note cependant que les plus faibles valeurs de pH de l’eau décantée de la filière no. 1 (figure 4) correspondent à des abattements moins de MES (figure 7).

À l’essai 4B, les valeurs de pH et de l’alcalinité sont légèrement supérieures à celles obtenues dans la filière no. 1 (AL/CH). Les dosages apparaissant à la figure 3 indiquent, pour la filière no. 1, un dosage d’alun inférieur de 16,2 % et un dosage de floculant comparable ou légèrement supérieur à ceux appliqués dans la filière no. 2. Dans ces conditions de dosage, l’utilisation de la combinaison AL/CH permet l’atteinte de meilleurs abattements de la DCO. Meyer et Emden (2003) indiquent que l’abattement de la DCO, lors du traitement d’une eau usée municipale dans des étangs d’aération, est supérieur lors de l’ajout de chitosane en raison de l’effet de ce biopolymère sur le processus de nitrification. Dans le cadre des présents essais, l’abattement supérieur de la DCO observé se traduit dans les procédés biologiques comme les étangs.
Tel qu’exposé à la section Conditions de débits et phase d’ajustement des dosages, une augmentation de charge à l’afluent en trois phases consécutives est présente. Lors de ces phases, les résultats d’abattement, en plus de respecter les conditions de rejet, sont aussi demeurés stables. Le % d’abattement en MES de la filière utilisant le chitosane semble cependant plus sensible à la variation de charge à l’afluent. On note que ce facteur peut avoir une influence dans le rendement de l’abattage des MES.

**Effet du flocculant sur le dosage de coagulant.** Pour l’ensemble de la période d’essais, le dosage moyen de coagulant dans la filière no. 1 est inférieur de 24,8 % (38 mg/L versus 50,5 mg/L) à celui de la filière no. 2. Le dosage du flocculant dans les deux filières est similaire (0,32 mg/L versus 0,30 mg/L). On observe que l’utilisation du flocculant naturel de type cationique a un effet notable sur la réduction du dosage de coagulant. Malgré un dosage moindre de coagulant acide, le pH de l’eau décantée de la filière no. 1 se maintient dans la même plage de pH que celui de la filière no. 2. L’acide utilisé pour la solubilisation du biopolymère lors de sa préparation contribue donc significativement à l’acidification du milieu aqueux. Cette réduction du dosage de coagulant (AL) et l’utilisation du biopolymère, qui est de surcroît solubilisé à l’aide d’un acide organique, mettent en place plusieurs conditions favorables à la valorisation des boues générées. Mentionnons que la présence d’un acide organique, tel l’acide acétique, s’avère plus favorable au processus de digestion anaérobie et que la réduction du dosage de coagulant offre certains avantages. Il permet notamment de réduire : 1) la teneur en sulfates dans les boues à digérer pouvant contribuer à la formation de soufre dans le biogaz; et 2) la teneur en métaux dans les boues à disposer permettant d’augmenter leur potentiel de valorisation. De plus, l’utilisation d’un biopolymère naturel et biodégradable réduit la teneur en polymère de synthèse dans les boues. Ainsi des teneurs moindres de coagulant chimique et de polymère de synthèse dans les boues de station d’épuration offrent de meilleures perspectives pour leur valorisation. L’effet de la diminution du dosage de coagulant sur la formation des boues, l’efficacité de la digestion anaérobie et de la déshydratation des boues ainsi que l’évaluation des biosolides générés (qualité et quantité) durant cette période d’essais n’ont cependant pas fait l’objet de la présente évaluation.

**Conclusion**

Des essais à l’échelle réelle ont été réalisés dans les conditions de débits et charges à l’afluent de la station rencontrant les critères de conception des ouvrages. Lors de ces essais, des débits quotidiens et des dosages relativement stables ont été observés. Les résultats montrent que des performances comparables d’abattement de la DCO et des MES avec les deux types de flocculant (CH et PS) ont été obtenus. Les résultats ont montré que l’abattement du P tot est constamment plus élevé avec la combinaison AL/PS en raison d’un dosage de coagulant (AL) supérieur. La condition utilisant le même dosage de coagulant avec les deux types de flocculant, tel que réalisé en laboratoire, n’a pu être effectuée à pleine échelle. Ainsi l’abattement supérieur du P tot avec le CH obtenu à l’échelle laboratoire n’a pas été reproduit à pleine échelle. Étant donné que la durée des essais et les variations typiques observées dans les installations sont relativement courtes, les essais devraient être poursuivis afin d’évaluer d’autres périodes et conditions d’utilisation du chitosane. Finalement différents aspects économiques liés à l’utilisation des produits coagulants et flocculants nécessitent d’être documentés par une période d’essais plus longue.

**Remerciements**

Les auteurs adressent leurs remerciements à MM. Marc Allard et M. Richard Perron (Ville de Rosemère, Service de l’hygiène du milieu) et à MM. Arnold Blais et Don Barclay (Marinard Biotech) pour leur support.

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Evaluation of Plantain Peelings Ash Extract as Coagulant Aid in the Coagulation of Colloidal Particles in Low pH Aqua System

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The use of plantain peelings ash extract (PPAE) as a coagulant aid in a low pH water was evaluated in the present studies. Plantain peelings were collected, washed, dried, and ashed in a furnace. The ash was extracted using deionized water, and the chemical composition was examined using an atomic absorption spectrophotometer. Synthetic turbid water of varying turbidities (50, 100, 300 NTU) and varying pHs (2, 3, 4) were prepared by clay dispersion in deionized water. The optimum alum dosages for the coagulation of colloidal particles in different turbid waters of varying pHs were determined by method of continuous variation using the jar test procedure. The residual turbidities of the treated waters were determined, and the alum doses that gave the minimum residual turbidities were taken as the optimum dosage for the removal of colloidal particles. The use of PPAE as a coagulant aid with alum showed an improvement in the value of residual turbidities of the treated waters. Results obtained from the different studies showed that treated waters of lower residual turbidities were obtained from synthetic waters of higher initial turbidities and pHs. The pH of the treated water decreased with an increase in alum dosage, whereas an increase in the pH value was observed with the addition of PPAE as a coagulant aid. High correlation coefficient values ($r^2$) were obtained when the changes in pH (i.e., $\Delta$ pH = pHf –pHi) of the treated waters were correlated with alum and PPAE additions, and mathematical relationships were derived from the linear graph. Studies on the effect of flocculation time on residual turbidity showed that an optimum flocculation time of 30 minutes was attained, when alum was used alone, before redistribution and redispersion of the flocs was noticed. This phenomenon did not occur when PPAE was used as a coagulant aid.

Key words: plantain peel extract, ash, turbidity, alum, coagulant

Introduction

A large number of human settlements have developed along watercourses. The demand on these watercourses as a source of potable water and a means of disposing the waste generated by the community has greatly depleted the natural quality that such splendid water resources possessed. The indispensable and foremost position of water in man’s daily operations has left him with no option other than to recycle such waters for reuse. This has culminated in the development of varying sophisticated water and wastewater treatment processes to cater for his water needs (Oladoja 2003).

Coagulation-flocculation is one of the simplest and most cost effective unit operations in the treatment of water and wastewater. This process, which is operational in most treatment plants, usually entails the use of a coagulant for the removal of dissolved and suspended colloidal particles from the system. In performing this operation, the reaction conditions are optimized for optimum process performance. The efficiency of the coagulation-flocculation process varies based on the physical and chemical characteristics of the water and the operating conditions. Aluminum salts are the chemicals most commonly used together with synthetic polymers (Diaz et al. 1999). However, different studies have discussed several serious drawbacks of using the alum salts. For example, Alzheimer’s disease and other related problems have been associated with residual aluminum in treated waters (Pan et al. 1999; Schintu et al. 2000; Divakaran and Pillai 2001). Synthetic organic polymers have been used as effective coagulant aids in drinking water purification systems (Bratby 1980). However, organic polymers have potential limitations. Polymer formulations contain contaminants (such as residual monomers and other reactant and reaction by-products) from the manufacturing process that could potentially negatively impact human health. Polymers and product contaminants can react with other chemicals added to the water treatment process to form undesirable secondary products (Kawamura 1991; Bolto 1995; Lee et al. 1998; Ozacar and Sengil 2003a). Consequent upon this, the need to develop coagulant and coagulant aids from natural sources is a necessity.

Coagulant aids are used in coagulation-flocculation operations, mainly to improve the efficiency of the primary coagulant and reduce costs. The choice of a material as a coagulant aid is premised on the particular physical or chemical property of the colloidal particulates or the water on which the operation is to be performed. In recent time, a surfeit of naturally derived substances have been reported as coagulant aids in water and wastewater treatment. Aqueous extracts of the seed of *Moringa oleifera* has been investigated as a coagulant...
aid in water and wastewater treatment (Ndabigengesere 1995, Ndabigengesere et al. 1995; Ndabigengesere and Narasiah 1998). Other materials of plant origin that have been studied include tannin (Ozcar and Sengil 2000, 2002, 2003a, 2003b, 2003c); extracts of okra and nimali seeds (Al-Samawi and Shokralla 1996); extracts of *Prosopis julifora* and *Cactus latifaria* (Diaz et al. 1999); and chitosan and modified chitosan biopolymers (Pan et al. 1999; Ashmore and Hearn 2000; Huang et al. 2000; Divakaran and Pillai 2001; Bratskaya et al. 2002; Strand et al. 2003; Roussy et al. 2005).

In the treatment of wastewater with low alkalinity (e.g., acid mine drainage), irrespective of the colloid concentration, the use of a primary coagulant alone has been found to be ineffective since the pH of the medium will be too low to effect rapid flocculation of colloidal particles. Addition of slaked lime (CaOH) as a coagulant aid furnishes necessary alkalinity which provides the much needed effect. Lime is an undesirable coagulant aid because of the problems associated with the sludge produced from its use (i.e., high sludge production and handling difficulties). Its optimum dosing (pH 11) is also undesirable for use, prior to or during biological treatment, and would require a very high dosage if wastewater with a low pH is encountered. Owing to these facts and the need to improve on the economy of water and wastewater treatment operations, the present studies examined the use of the water extracts of plantain peel ash as a coagulant aid in the treatment of water of low pH values (e.g., acid mine drainage, sulphuric acid plant effluent).

The plantain peel ash extracts (PPAE) come from the peelings of the plantain fruit. The plantain, also known as cooking banana, is classified as *Musa paradisiaca*. Banana is a common name for any of a genus of tropical, treelike herbs, and their fruits. Bananas make up the genus *Musa* of the family *Musaceae*. Species of the genus are native to Southeast Asia, but are now grown extensively in all tropical countries for their fruits, fiber, or foliage. The banana is a large, herbaceous plant with a perennial root or rhizome from which the plant is perpetuated by sprouts or suckers. The fruit vary in length from about 10 to 30 cm. The fruit of the plantain is larger, coarser, and less sweet than the banana that is generally eaten raw. The edible part of the banana contains, on the average, 75% water, 26% carbohydrate, and about 1% each of fat, protein, fiber, and ash. Other parts of the plant abound in fiber which can be used in the manufacture of paper and cordage. Owing to the rich concentration of alkali in the water extracts of plantain peelings ash, the making of soap from the ash-derived alkali is an age-old craft in most West African countries. In the present studies, the experiments were conducted in duplicate and the results were reproducible within values of 0.1 to 0.5 NTU. The experiment involved the addition of the coagulants to turbid water (500 mL) of a particular pH. This was followed by rapid mixing of the mixture (i.e., coagulant plus turbid water) for 2 minutes at 200 rpm, and slow stirring for 2 minutes at 45 rpm. The mixture was allowed to settle and samples were withdrawn from a 3-cm depth after 20 minutes for turbidity and pH determinations.

### Extraction of Plantain Peelings Ash

Plantain peelings were obtained from a commercial farm in Nigeria. They were dried in the oven at 103 to 105°C. The dried peelings were placed in a furnace and heated at 550 to 600°C to obtain the plantain peelings ash. The ashed sample was homogenized and sieved to remove large particle size fractions. Plantain peelings ash (150 g) was placed in 2.5 L of deionized water and kept at 60°C in a thermostated water bath for 8 h. Subsequently, the slurry was filtered to obtain the extract. The molarity of the PPAE was determined by titration against 0.1 M HCl using a phenolphthalein indicator. Metallic ion content of the extract was determined using an atomic absorption spectrophotometer (AAS).

### Synthetic Turbid Water

A stock of the synthetic turbid water sample was prepared by adding a known quantity of pulverized clay to a known volume of deionized water. The mineralogy and geochemistry of the clay sample was studied using a Diano 2100 E-X-ray diffractometer and an AAS, respectively. Synthetic water, of different turbidities (50, 100, and 300 NTU), was prepared from the stock by dilution with deionized water. The pH of the turbid water was adjusted with dilute HCl and NaOH to give the desired pHs (2, 3, and 4).

### Alum Solution

An accurately weighed quantity of alum [Al₂(SO₄)₃·18H₂O] (Merck) was dissolved in distilled-deionized water to obtain a final alum concentration of 0.1 g of alum per mL. A fresh alum solution was prepared daily for reliability of results.

### Jar Test Experiment

The different studies, concerning the coagulation and flocculation of the synthetic turbid water, were conducted using the jar test method. The optimum alum dosages for the coagulation of different turbid waters (50, 100, and 300 NTU) at different pHs (2, 3, and 4) was determined. The ability of PPAE to act as a coagulant aid in the treatment of water with low alkalinity was also assessed. Each experiment was conducted in duplicate and the results were reproducible within values of 0.1 to 0.5 NTU. The experiment was conducted in a system containing six rectangular pales (75 x 25 cm). A typical experiment involved the addition of the coagulants to turbid water (500 mL) of a particular pH. This was followed by rapid mixing of the mixture (i.e., coagulant plus turbid water) for 2 minutes at 200 rpm, and slow stirring for 20 minutes at 45 rpm. The mixture was allowed to settle and samples were withdrawn from a 3-cm depth after 20 minutes for turbidity and pH determinations. The
effects of flocculation time on the residual turbidities of the different turbid waters at different pHs were studied at the optimum dosage of alum and alum/PPAE combination ratio.

Results and Discussion

PPAE Composition

The physicochemical characteristics of the PPAE are presented in Table 1. The alkaline nature of the water extracts of the plantain peelings could be seen in the pH value of the extract (11.08). The molarity of the extract, determined by the titration of the extracts against 0.1 M HCl, using a phenolphthalein indicator, was 0.654. The turbidity of the water extract, determined with the aid of a turbidimeter, was 6.0 NTU. The results of the AAS analysis revealed that the principal metallic ions were potassium (87.32%) and sodium (11.08%). Other metallic ions, found within the detectable limits of the AAS, were present in relatively small quantities.

Clay Characteristics

An X-ray diffractometer (Diano 2100*E) was used for the clay mineralogical analysis. A copper anticathode (λ = 1.54Å) was used. XSPEX version 5.41 software was used in the interpretation of the diffractogram. The interpretation of the diffractogram obtained from the X-ray diffraction analysis revealed the presence of the following clay minerals: kaolinite (27.68%); smectite (7.55%); illite (16.43%); mixed layer (i.e., smectite/illite mixed layer) (8.48%); and quartz (39.86%). Geochemical analysis of the different clay samples was performed using AAS after the clay samples were digested in a polypropylene bottle using a mixture of HF, HCl, and HClO4. Ten major elements were determined. The geochemical analysis showed the abundance of the presence of SiO2 (50.11%), Al2O3 (17.00), and structural water (H2O+) (15.01%). This revealed the hydrated

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>K2O</td>
<td>87.32%</td>
</tr>
<tr>
<td>Na2O</td>
<td>11.08%</td>
</tr>
<tr>
<td>CaO</td>
<td>1.35%</td>
</tr>
<tr>
<td>CrO</td>
<td>0.08%</td>
</tr>
<tr>
<td>ZnO</td>
<td>0.01%</td>
</tr>
<tr>
<td>Fe2O3</td>
<td>0.06%</td>
</tr>
<tr>
<td>PbO</td>
<td>0.04%</td>
</tr>
<tr>
<td>NO2</td>
<td>0.06%</td>
</tr>
<tr>
<td>pH</td>
<td>11.08</td>
</tr>
<tr>
<td>Molarity</td>
<td>0.65 M</td>
</tr>
<tr>
<td>Turbidity</td>
<td>6.0 NTU</td>
</tr>
</tbody>
</table>

Fig. 1. Determination of optimum alum dose at (a) 300 NTU, (b) 100 NTU, and (c) 50 NTU.
aluminosilicate nature of this material. The percentage oxide compositions of the other elements present was: Fe₂O₃ (1.42%); MgO (78%); CaO (6.01%); Na₂O (1.61%); K₂O (1.02%); TiO₂ (0.21%); MnO (0.001%); and P₂O₅ (0.01%).

**Optimum Alum Dose Determinations**

The optimum alum doses, which are the smallest doses giving the best turbidity removal, were determined for each of the turbid waters (50, 100, and 300 NTU) at different pHs (2, 3, and 4). The experiments were conducted in a jar test apparatus. Figure 1 shows the results of the different experimental runs. The turbidities of the different turbid waters reduced with increasing alum dosages. The reduction in the turbidity values continued until a minimum turbidity value was obtained, thereafter an increase in the turbidity value was observed. The smallest doses that gave optimum turbidity removal for the different turbid waters varied with the initial turbidity of the water. The higher the initial turbidity, the higher the value of the optimum alum dose used (3.2 mL/L for 300 NTU, 2.8 mL/L for 100 NTU, and 2.0 mL/L for 50 NTU). The residual turbidities, at lower initial turbidities, were higher than when water of higher turbidity of identical pH was used in the experimental runs.

The observed difference in the value of the percentage turbidity removal in the different turbid waters could be ascribed to the different mechanisms of coagulation, as proposed by Özçakar and Sengil (2002). At higher turbidities, the predominant mechanism for coagulation of colloidal particles was “sweep coagulation.” The large quantity of Al(OH)₃⁺⁺ precipitate was capable of sweeping the fine colloidal particles from the water. The Al(OH)₃ adsorbed on the surface of the particles and reduced the negative charge with its slightly positive charge. When relatively low turbid water was used (i.e., 50 and 100 NTU), the effectiveness of this coagulation may not have been as high as when water of high turbidity was used.

The results obtained, when the effect of initial pH of the turbid water was taken into consideration, showed that the values of the optimum alum dose were similar, but the residual turbidity values varied with the initial solution pH. Synthetic turbid water of higher initial pH value had lower residual turbidity values than those of lower initial pH values. The initial solution pH is one of the important factors that is given higher consideration in the use of coagulation-floculation in the water industry. This could be understood from the reported speciation of alum in the water. Alum dissociates in water to give Al³⁺, SO₄⁻² and various alum complexes such as Al(OH)⁵⁻, Al(OH)₃⁻, and Al(OH)₉⁻, depending on the pH of the medium. The various positively charged species that are formed may combine with negatively charged colloids to neutralize part of the charge on the colloid particles (Ademoroti 1996). The colloidal materials then come together and become incorporated into masses that can be readily precipitated. It is noteworthy that the pH of the water plays a prominent role in the determination of the hydrolysis species that is predominant in the aqueous medium. Lower pH favours the species with higher positive charge on them. At pH below 5.0, the OH⁻ is insufficient to precipitate Al³⁺ completely, so that [Al(OH)₅⁻] and Al(OH)₉⁻ occur. The positively charged Al attracts the colloidal particles and forms loose flocs which are not dense enough for easy macrofloc formation and subsequent sedimentation.

Gregor et al. (1997) reported that charge neutralization is the mechanism used to explain the precipitation of natural organic matter in operational regions where aluminum hydroxide precipitation of natural organic matter is minimal (i.e., low pH). Greenwood and Earnshaw (1989) posited that the ionic mobility of H⁺ ions is much faster than any metallic ion in solution. The authors were of the opinion that the extremely fast ionic mobility of the H⁺ ions can affect the operational pHs in the present studies (i.e., low pH), could be a source of interference in the potency of the positively charged alum species in charge neutralization, which could be the domineering mechanism, of the negatively charged colloidal particles.

**Studies on the Use of PPAE as a Coagulant Aid**

Metal salt coagulants react with the alkalinity in water to produce insoluble metal hydroxide precipitates that enmesh the colloidal particles in water and adsorb other materials including dissolved organic matter present (Gray 1999). Where the natural alkalinity of the water is insufficient, lime in the form of calcium hydroxide is added to aid the formation of flocs. In order to assess the ability of PPAE to function as a coagulant aid in the treatment of low alkalinity water, half of the quantities of the optimum alum doses obtained from the previous studies (see section above: Optimum Alum Dose Determinations) were used with varying quantities (in millilitres) of the PPAE (0.15, 0.20, 0.25, 0.50, 1.00, and 1.20). The results presented in Fig. 2 show the effect of the addition of PPAE, as a coagulant aid, on the residual turbidities of the treated waters. The values of the residual turbidities of the treated synthetic waters were lower with the addition of PPAE than when alum was used alone. The water with the initial highest turbidity value (i.e., 300 NTU) produced water with the highest value of percentage turbidity removal (93.33% at pH 4; Table 2). This could be ascribed to the high colloid concentration in the medium, which promotes interparticle bridging and macrofloc formation. At relatively low colloid concentrations (i.e., 100 and 50 NTU), an improvement in the value of the residual turbidity was also recorded when the results obtained from these studies were compared with studies where alum alone was used.

The importance of the natural alkalinity of water to be treated, in the efficacy of coagulation-floculation operations, is a fact that has been recognized in the water industry. This is because at pH levels below 5.0, OH⁻ is insufficient to precipitate Al³⁺ completely so that
Plantain Peeling Ash Extract as Coagulant Aid

Al(OH)\(^{2+}\) and Al(OH)\(^{3+}\) occur. Ademoroti (1996) surmised that the residual alkalinity serves to buffer the systems at pH levels above 5.0 for Al\(^{3+}\) and above 4.0 for Fe\(^{3+}\) to ensure complete precipitation of the coagulation ions.

Beer and Gibbs (1975) pointed out that iron(III) chloride, added to a slightly alkaline effluent, dissociates into iron(III) hydroxide ions and chloride ions. The iron(III) hydroxide ion, having a positive charge, attracts colloidal particles and forms a group of flocs. On the addition of a suspension of slaked lime, the more alkaline conditions cause the loose flocs to form into dense flocs which settle out rapidly. If this scenario is applied to the application of PPAE, which is alkaline (pH=11.08) and predominantly KOH, the changes occasioned by the addition of the extract could be represented thus:

\[
\begin{align*}
\text{Al}_2(\text{SO}_4)_3 + 6\text{H}_2\text{O} & \rightarrow 2\text{Al(OH)}_3 \cdot 2\text{H}_3^+ + 3\text{SO}_4^{2-} \\
2\text{Al(OH)}_3 \cdot 2\text{H}_3^+ \rightarrow \text{colloidal particle} & \rightarrow \text{loose flocs} \\
2\text{Al(OH)}_3 \cdot 2\text{H}_3^+ + 6\text{KOH} & \rightarrow 2\text{Al(OH)}_3 + 6\text{H}_2\text{O} + 6\text{K}^+ \\
\text{Al(OH)}_3 + \text{colloidal particle} & \rightarrow \text{dense flocs}
\end{align*}
\]

Despite the observed improvement in percentage turbidity removal at 100 and 50 NTU when the PPAE was used, the percentage turbidity removal was relatively low when the results were compared with synthetic water of 300 NTU. The relatively low initial turbidities of the waters could inhibit the rapid formation of flocs as the rate of interparticle contact is too slow to utilize destabilization by charge neutralization.

In addition to the determination of the turbidity of the treated water, the pH value was also determined and the results obtained are presented in Fig. 3. The pH values of the treated water, when alum was used alone and when PPAE was used as a coagulant aid, were compared. It was noted that when alum was used alone, the pH of the treated water was lower than the initial pH of the turbid water. This same trend was observed in all the studies carried out using alum alone (i.e., water of different turbidities and pHs). When alum was used with the coagulant aid (i.e., PPAE), the pH values of the treated waters were higher than the respective initial pHs. The observed pH elevation increased with an increase in PPAE dosage. The dissolution of alum in water furnishes the aqua medium with H\(^+\), which depresses the pH of the medium. Owing to the paucity of OH\(^-\) ion in a low alkalinity water, the neutralization of the H\(^+\) ion produced is hindered, hence acidity prevails. When alum

### Table 2. Percentage (%) turbidity removal in the use of PPAE as coagulant aid with 0.5 optimum alum doses

<table>
<thead>
<tr>
<th>PPAE dose (mL/L)</th>
<th>300 NTU</th>
<th>100 NTU</th>
<th>50 NTU</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 4</td>
<td>pH 3</td>
<td>pH 2</td>
</tr>
<tr>
<td>0.15</td>
<td>78.33</td>
<td>76.67</td>
<td>64.33</td>
</tr>
<tr>
<td>0.20</td>
<td>84.00</td>
<td>80.33</td>
<td>76.33</td>
</tr>
<tr>
<td>0.25</td>
<td>90.00</td>
<td>86.67</td>
<td>78.67</td>
</tr>
<tr>
<td>0.50</td>
<td>93.33</td>
<td>91.67</td>
<td>86.67</td>
</tr>
<tr>
<td>1.00</td>
<td>84.00</td>
<td>84.67</td>
<td>89.33</td>
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<tr>
<td>1.20</td>
<td>80.33</td>
<td>83.33</td>
<td>77.67</td>
</tr>
</tbody>
</table>

Fig. 2. Effect of PPAE as a coagulant aid at (a) 300 NTU, (b) 100 NTU, and (c) 50 NTU.
is used with the PPAE, the PPAE used acts as a reservoir of OH\textsuperscript{-} ions, thereby reducing the effect of the H\textsuperscript{+} ion generated by the dissolution of alum, and the acidity effect is suppressed. This accounts for the observed pH elevation when alum was used with the PPAE.

The changes in the pH (ΔpH) of the treated water at different alum dosages, and alum and coagulant aid (PPAE) dosages, were regressed (Fig. 3a and b) to derive a mathematical relationship with high coefficients of determination ($r^2 > 0.940$). Therefore, ΔpH can be expressed as a function of alum and PPAE dosage at different pHs as follows:

- **Alum:**
  - pH\textsubscript{4}: $\Delta$ pH = 0.1661A\textsuperscript{0.1318}
  - pH\textsubscript{3}: $\Delta$ pH = 0.1628A\textsuperscript{0.132}
  - pH\textsubscript{2}: $\Delta$ pH = 0.208A\textsuperscript{0.1421}

- **PPAE:**
  - pH\textsubscript{4}: $\Delta$ pH = 2.9715P\textsuperscript{0.3773}
  - pH\textsubscript{3}: $\Delta$ pH = 1.4535 P\textsuperscript{0.2206}
  - pH\textsubscript{2}: $\Delta$ pH = 0.6975P\textsuperscript{0.1264}

**Effect of Flocculation Time**

The time of macrofloc formation (flocculation time) is one of the operating parameters that is given great consideration in any water treatment plant that involves coagulation-flocculation operations. Consequent upon this, the effect of flocculation time on the residual turbidities of the treated water was studied at the optimum dosage of alum and alum/PPAE combination ratio. The flocculation time was varied between 10 and 90 minutes. The results obtained are presented in Fig. 4. When only alum was used, the residual turbidity of the treated water reduced, with time, until after 30 minutes when

![Fig. 3. Linear correlation of $\Delta$pH = pH\textsubscript{i} - pH\textsubscript{j} versus (a) alum dosage and (b) PPAE dosage.](image1)

![Fig. 4. Effect of flocculation time on residual turbidity at (a) 300 NTU, (b) 100 NTU, and (c) 50 NTU.](image2)
a minimal increase in the value of residual turbidity was noticed. Nozaki et al. (1993), Sengil (1995), and Ozacar and Sengil (2002) have reported an optimum flocculation time of 30 minutes when natural polyelectrolyte, alunite, and tannin were used, respectively, as a coagulant and coagulant aid in water treatment. Sengil (1995) ascribed the increase in the residual turbidity after the optimum time to the possibility of redispersion and restabilization of flocs at higher flocculation time. This problem of redispersion and restabilization did not arise when the PPAE was used as a coagulant aid with alum; instead, a reduction in the value of the residual turbidities was recorded over the entire period of study (90 minutes).

**Conclusion**

The efficacy of PPAE as a coagulant aid in a low pH aqua system was examined. Water extract of the plantain peelings ash was prepared, and the physicochemical characteristics were studied. The results of the characterization showed that the PPAE was alkaline (pH 11.08) and the molarity was 0.65 M. The principal metallic ions in the extract were potassium (87.32%) and sodium (11.087%). The ability of PPAE to function as a coagulant aid was shown by the higher percentage of turbidity removal observed when waters of same characteristics (i.e., initial turbidity concentration and initial pH) were subjected to the same treatment procedure. The percentage turbidity removal varied with the initial pH and turbidity. The higher the initial turbidity of the synthetic water, the higher the percentage turbidity removal observed. The lower the pH, the higher the residual turbidity of the treated water observed.

When the pH values of the treated waters were compared, a diminution in the value of the initial pH was observed in the water treated with alum alone. The use of PPAE as a coagulant aid produced waters with pHs higher than the initial pHs. The observed changes in pH of PPAE as a coagulant aid produced waters with pHs observed in the water treated with alum alone. The use of PPAE as a coagulant aid with alum; instead, a reduction in the value of the residual turbidities was recorded over the entire period of study (90 minutes).

**References**


Received: 30 April 2007; accepted: 20 March 2008.
Optimization of Solids Separation in Dissolved Air Flotation

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Sizes of flocs were analyzed to identify characteristics of the particle size distribution optimal for separation by dissolved air flotation (DAF). Optical microscopes and two particle counters were used for floc sizing. A Brightwell Technologies particle counter was found to provide floc size measurements in agreement with improved microscopic methods. The particle counter provided distribution of flocs with sizes down to 1 micron (μm). This allowed for inclusion of flocs with size ranging from 5 to 1 μm, which were excluded from the analyses in the earlier study. Four alum dosages were applied: 15, 25, 40, and 60 mg/L. The turbidity and colour of the DAF effluent at alum dosages of 25, 40, and 60 mg/L were very similar. However, the analysis of the flocs in the treated effluent revealed that, at the alum dose of 60 mg/L, particle removal was the best. Therefore, this dosage was selected as optimal for the solid/liquid separation process. The average size of coagulation flocs at 60 mg/L was approximately 30 μm, and was equal to the estimated size of air bubbles produced by the saturator. Therefore, this study confirms the finding of the earlier work claiming that the optimum DAF performance is attained when the mean floc size and the bubble size are equal. Similar size of floc and bubble indicates that flocs act predominantly as nuclei for bubble formation. This finding contributes to the knowledge of mechanisms of floc air bubble attachment in DAF.

Key words: flocs, air bubbles, dissolved air flotation (DAF), microscope-particle counter

Introduction

Chemical coagulation precedes dissolved air flotation (DAF) in the water treatment process. Therefore, the efficiency of the DAF clarification unit is largely determined by the coagulation conditions.

Coagulation and Flocculation Before DAF

In water treatment, flotation is not successful without coagulation. Two conditions are necessary for favourable particle flotation in water treatment: (1) particle charge neutralization and (2) particle hydrophobicity (Edzwald 1995). Addition of appropriate doses of chemicals (coagulant) is the most common method for particle charge neutralization. Bubble attachment to particles requires hydrophobic particle surfaces or hydrophobic spots on particles (Goochin and Solari 1983). Freshly precipitated or amorphous aluminium hydroxides have polar surface groups and are hydrophilic; however, this hydrophilic effect may be reduced by charge neutralization.

Review of Floc Sizes in DAF Process

Different opinions still exist over the optimum floc size for the DAF process and the coagulation conditions related to it. For example, Edzwald et al. (1992) emphasized that long flocculation was not needed, and pin-floc sizes between 10 to 30 μm were most favourable. In his study, the flocculation time of 5 to 15 minutes and a ferric chloride dosage as low as about 25 mg/L was sufficient for successful DAF operation. Edwald et al. (1992) measured the floc size with a particle counter operating on the light blocking principle. The flocs with diameters ranging from 2 to 120 μm were measured. The average floc diameter for flocculated water was about 15 μm. Although Tambo (1979) and Gorczyca and Ganczarczyk (1995) reported presence of flocs larger than 120 μm at coagulant dosages applied by Edwald et al. (1992), these large particles were not included in the average floc size calculations. Consequently, the reported average floc size could have been underestimated. The details on the floc sampling and sizing methods are also not provided; for example, it is not clear how ‘floc diameter’ was calculated.

Fukushi et al. (1995) suggested that larger flocs are preferred for the DAF process. Fukushi et al. (1995) measured flocs using a microscope with video photography in a specially designed flow cell. Again, the details on the floc sizing technique are not provided. For example, the authors claim to be able to identify flocs with sizes ranging from 1 to 1,000 μm. Yet, many earlier studies that used the same floc sizing technique were not able to identify flocs smaller than 20 μm (Tambo 1979; Gorczyca and Ganczarczyk 1995). The floc size is also not defined in the paper.

Vlaski et al. (1997) measured sizes of flocs formed in FeCl₃ coagulation prior to DAF. Natural waters spiked with cyanobacteria were used in laboratory and pilot scale DAF experiments. The mean size of flocs at the optimum dose of 10 mg of Fe (III) per litre was about 25 μm. The saturator operated at the pressure of 5 bar (505 kPa) and a 5% recycle ratio. The authors concluded that small, shear-resistant flocs are not necessary for efficient DAF. Larger flocs, formed at a low flocculation velocity

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gradient (G) floated as well as the small flocs produced at high G values. Compared with the laboratory scale studies, larger G values had to be applied at the pilot test. This increased flocculation energy input was necessary to create better contact opportunities for the particles in the larger water volumes used in the pilot studies.

Han et al. (2007) measured sizes of particles at several full-scale DAF water treatment plants operating in Korea. A laser particle counter was used. The reported size of particles varied from 1 to 100 μm, with logarithmic averages between 20 to 30 μm.

Sizes of Air Bubbles in DAF

In the DAF unit, typically 5 to 10 % of the raw or treated water is saturated with air under pressure (414 to 586 kPa). The saturated water (recycle) is introduced into the front of the DAF tank by means of specialized nozzles and consequently undergoes a pressure drop. The pressure change results in the formation of small bubbles with diameters between 10 to 100 μm, with most of the bubbles with sizes between 40 to 80 μm (Edzwald 2007).

The operating pressure of the saturator is the main factor affecting bubble size (Han et al. 2002). Han et al. (2002) measured bubbles formed at different saturator pressures using a Laser Trac PC 2400 D particle counter and a microscope coupled with an image analysis system. The reported bubble sizes ranged from 10 to 100 μm. The saturator pressures varied from 2 to 6 atm. The mean bubble size at the saturator pressure higher than 3.5 atm (353 kPa) remained steady at about 30 μm.

Mechanisms of Floc Bubble Attachment and Relationship of Floc and Bubble Sizes

The bubbles may attach to the suspended particles and cause them to float to the surface of the water. The exact mechanism of floc-bubble attachment is not well understood. The preformed bubbles may adhere to a preformed floc, or may become entrapped within the floc. The floc particle may also act as nuclei for bubble formation (Crittenden et al. 2005).

In the first two mechanisms, the bubbles are much smaller than the floc. In the third mechanism, the bubble is about equal to the floc size.

Park et al. (2001) reported that most efficient bubble-particle collision was achieved when the average floc size was close to the bubble size. It was also shown that particles with diameters smaller than 20 μm were not removed easily in the DAF process (Han et al. 2002); the reported removal of these particles was only 10% at a mean bubble size of about 40 μm.

Preliminary Studies with the City of Winnipeg Tap Water

Gorczyca and Zhang (2007) conducted a bench-scale continuous flow DAF experiment using Winnipeg tap water. Three different dosages of alum were applied: 15.5, 25.5, and 41.7 mg/L. Sizes of flocs formed at different coagulant dosages were analyzed to identify characteristics of the particle size distribution optimal for separation by flotation. An alum dose of 25.5 mg/L was found to be optimal based on the best treated effluent.

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**TABLE 1. Operating conditions of pilot and bench scale DAF unit**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pilot plant study results*</th>
<th>Bench studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw water flow rate (L/min)</td>
<td>-333</td>
<td>1.63</td>
</tr>
<tr>
<td>Raw water temperature (°C)</td>
<td>4–15</td>
<td>7–11</td>
</tr>
<tr>
<td>Alum dosage (mg/L)</td>
<td>60 a</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>41.7</td>
</tr>
<tr>
<td>Coagulation time (sec)</td>
<td>In-line mixing</td>
<td>120</td>
</tr>
<tr>
<td>Flocculation time (min)</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>DAF hydraulic loading rate (m/hr)</td>
<td>10–20</td>
<td>2.2</td>
</tr>
<tr>
<td>DAF saturator pressure (psi)</td>
<td>70–80</td>
<td>90</td>
</tr>
<tr>
<td>Recycle ratio (%)</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Effluent pH</td>
<td>6.6</td>
<td>6.4–6.8</td>
</tr>
</tbody>
</table>


a With 2 mg/L of CatFloc 2 polymer and pH adjustment.
turbidity of 0.25 Nephelometric Turbidity Units (NTU) and colour of 3.8 True Colour Units (TCU). At this dose, alum coagulation flocs had the average size of 37 μm. The bubble sizes were not measured in this early study. The average size of the air bubbles in the DAF unit was assumed to be 30 μm based on the bubble measurement conducted by Han et al. (2002) at the same saturator pressure. Therefore, the main conclusion of the Gorczyca and Zhang (2007) study was that the best DAF effluent quality can be attained when floc and bubble size are equal. This finding confirmed the relationship between the floc and the bubble reported earlier by Park et al. (2001) and suggested that it may be true for other water matrices.

Size of the coagulation flocs in this early study was estimated with a microscope using 4 x objectives (40 x magnification). Flocs smaller than 5 μm could not be detected under this magnification and were therefore excluded from the analyses. As a result of this exclusion, the calculated mean floc size may have been overestimated. Also, the average floc sizes were calculated from the measurements of about 200 randomly selected flocs only. This sample size was not statistically designed; it was simply based on the sample size used in the earlier floc sizing studies (Li and Ganczarczyk 1986; Gorczyca and Ganczarczyk 1995).

The operating conditions of the bench-scale DAF unit, such as coagulation/floculation time, alum dose, saturator pressure, and recycle ration, were based on the pilot-scale DAF unit operated by the City of Winnipeg (Table 1). Yet, the optimum coagulant dosage identified in the pilot study, which is 60 mg/L of alum, was not even tested in the earlier bench-scale study. There were several other differences between the pilot- and bench-scale units used in the study of Gorczyca and Zhang (2007). The bench-scale unit operated at longer coagulation/floculation detentions times and much lower hydraulic loading rates compared with the pilot-scale unit. The effects of these differences were discussed in details elsewhere (Gorczyca and Zhang 2007).

Objectives of this Study

The purpose of this study was to continue earlier research on determining the optimum floc sizes for the DAF separation initiated by Gorczyca and Zhang (2007). The bench-scale DAF apparatus used in the early study was modified to bring the coagulation/floculation conditions closer in line with the parameters used in City of Winnipeg pilot DAF plant research. Also in this study, the applied alum dose range was increased up to 60 mg/L to include optimum dosages identified during the City of Winnipeg pilot studies.

Another purpose of this study was to determine the reliability of the particle counter for providing accurate measurement of floc sizes. The flocs were sized using two techniques, microscopes, and two particle counters to improve the precision of the mean size estimate. This improved floc sizing technique permitted inclusion of flocs in the range of 1 to 5 μm in the distribution. These flocs were not measured in the earlier study. In this study, particles in the treated effluents were also analyzed to estimate particle removal efficiency in the DAF process.

Experimental Methods

Water Source

All the experiments were conducted using the tap water at the Environmental Laboratory of the University of Manitoba, Winnipeg, Manitoba (Canada).

City of Winnipeg water supply. Drinking water in Winnipeg comes from Shoal Lake, located on the border between Manitoba and Ontario. It is transported via an aqueduct to Deacon Reservoir, an 8,400 million litre reservoir located on the eastern edge of the city. Deacon Reservoir supplies three smaller reservoirs in the city, which, in turn, supply the distribution system. The treatment and storage system currently used in Winnipeg is shown below in Fig. 1. The water treatment system is currently very simple, consisting only of chlorination. In addition to chlorination, fluoride and orthophosphates are added to the water prior to distribution to the public. Beyond this chemical dosing, there are no other treatment procedures (Winnipeg Water Consortium 2001).

The quality of Winnipeg tap water, which is essentially chlorinated raw lake water, is generally within the Canadian Drinking Water Quality Guidelines (Health Canada 2007), but there are several areas in which performance is poor (Table 2). Turbidity can be as high as 2.6 NTU, while the guideline recommends a value below 0.3 NTU. High total organic carbon (TOC) levels in the range of 4 to 17 mg/L are present. Due to the high dose of chlorine (about 7 mg/L), the trihalomethane (THM) concentration ranges from 50 to 205 mg/L, and is frequently over the guideline of 100 ppb (Health Canada 2007). High algae levels in Deacon Reservoir or Shoal Lake are the cause for noticeable taste and odour of the tap water.

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Fig. 1. Current drinking water supply system in Winnipeg.
A pilot-scale water treatment plant (WTP) was designed to define a state-of-the-art and cost-effective water treatment process for the City of Winnipeg. It was operated continuously from June 1996 through the spring, summer, fall, and winter Shoal Lake water quality seasons (16 months). Direct filtration and DAF processes were investigated in the pilot studies. The finished water quality goals used in the evaluation of the pilot study performance are listed below:

- Filtered water turbidity, <0.1 NTU;
- Concentration of particles (>2 μm), <20 particles/mL;
- TOC removal, >40%;
- Taste and odour control, <10 Threshold Odour Number;
- Filter water production rate, >200 m³/m².

The DAF process was found to be superior in all the experimental categories investigated. A four-step water treatment process was developed and recommended for Winnipeg (Fig. 2): (1) DAF for suspended solids, mainly algae removal; (2) ozonation for primary disinfection and taste and odour control; (3) biological activated carbon (BAC) filters as a secondary barrier for pathogen and organics removal; and (4) chloramination for disinfection throughout the distribution system (Winnipeg Water Consortium 1999).

**Bench-Scale DAF Apparatus**

The bench-scale DAF apparatus used in this study has been described in detail in the earlier study (Gorczyca and Zhang 2007). In this study, several modifications to the operating conditions of the unit were made to match the conditions in the pilot plant. A schematic diagram of the apparatus is presented in Fig. 3.

The water depth was decreased from 19.3 cm in the earlier study to 8.7 cm here to achieve a shorter coagulation time. After the raw influent and alum were mixed in the rapid mix chamber, the process water passed to the first of two flocculation tanks. The water depth in the flocculation tanks was decreased, from 39 cm in the earlier study to 26 cm here, to achieve the lower flocculation time of 10 min. Also in this study, the applied alum dose range was increased up to 60 mg/L to include optimum dosages identified during the City of Winnipeg pilot studies. Comparison of the operating conditions of City of Winnipeg pilot- and the bench-scale units used in this and the earlier study is shown in Table 1.

The hydraulic loading rate of the bench-scale DAF unit was 2.3 m/hr, which is much smaller than the typical values in most full-scale DAF units. However, this low hydraulic loading rate is a constraint of the geometry of the apparatus used. Since the flocculation time and hydraulic loading rate in the flocculation chamber are both

![Fig. 2. Schematic diagram of proposed water treatment plant for the City of Winnipeg.](image-url)
controlled by the flow rate, a higher hydraulic loading rate of 10 to 20 m/hr, more in line with the pilot plant study, would have reduced a flocculation time to only 1 to 2 minutes.

Bench-Scale DAF Operation

The bench scale experiments were conducted in March 2006. Tap water from the Environmental laboratory at the University of Manitoba was used as the raw water.

The DAF treated effluent was analyzed for turbidity, colour, and pH every 30 minutes during each test run. The samples for particle analysis were collected only when the best and most steady DAF effluent quality was reached. Particles suspended in the raw water were fixed on glass slides using the procedure described elsewhere (Gorczyca and London 2003). Five replicate glass slide samples were prepared from raw water.

The floc samples were collected near the effluent of the second flocculation tank. The sampling point for coagulated samples is shown in Fig. 4 by the arrow. Approximately 1-mL samples containing flocs were withdrawn using a large-mouth pipette and placed in a Petri dish. Three replicate Petri dishes were prepared for each coagulant dosage. The samples were diluted with tap water to prevent the flocs from coming into contact with each other in the Petri dish.

DAF effluent samples were collected from the spigot as shown in Fig. 5. The sludge was skimmed off from the water surface manually at 15-minute intervals.

Particle Size Measurements

Microscopic analysis. Microscope particle size analysis was performed for the influent raw water and coagulated water; however, only sizes of coagulation flocs were used in the treatment performance evaluation. A standard trinocular polarizing microscope (Nikon 400) was used to view the influent samples, while a stereoscopic microscope (Nikon SMZ 800) was used to view the coagulated samples.

Fig. 3. Bench-scale DAF apparatus used in this study.

Fig. 4. Bench-scale DAF apparatus: flocculation tanks.
Particles in raw water. Samples of raw water fixed on a glass slide were photographed under 400 x magnification (40 x objective lens) using a Nikon 400 microscope connected to a digital microscope camera (Olympus DP70). The images of particles were saved for further measurements with an image analysis system, Image Pro Plus version 4.5 (Media Cybernetics Inc. 2002). For particle image detection and sizing, an automatic mode, was chosen. The system was calibrated according to the procedure described in the image analysis system manual. The projected area (cross-sectional area) of the particles was measured and the equivalent diameter was calculated as follows:

\[ \text{Equivalent diameter} = \left( \frac{4 \cdot \text{Projected area}}{\pi} \right)^{1/2} \]  \hspace{1cm} (1)

To obtain representative distribution of particle and flocs from the microscopic analyses, all particles identified on 20 randomly selected fields of views were measured (Parker 1972). Particles with equivalent diameters ranging from 1 to 70 μm could be identified at 400 x magnification. Particle measurement techniques are described in more detail elsewhere (Gorczyca and London 2003; Gorczyca and Zhang 2007).

Coagulation flocs. The stereoscopic microscope allowed direct observations of coagulation flocs without agar embedding. Alum flocs are almost translucent, and small flocs blend in well with an agar background. Flocs were measured at a magnification of 63 x using the same procedure as for raw water particles. Flocs with equivalent diameters ranging from 3.5 to 350 μm could be measured at this magnification.

Particles in DAF effluent. The particles in the DAF treated effluent were not analyzed with the microscopes for reasons explained later.

Particle Counter Analyses

Two particle counters were used to analyze particles in tap water, coagulated water, and DAF effluent. The Spectrex PS-2200 uses laser light scattering technology, and it can be used to measure particle size and concentration in a sample automatically. About 25 mL of a floc-containing sample was placed in the measurement compartment. Before each reading, the sample was gently agitated for 30 seconds.

The Brightwell Technologies Dynamic counter (DPA 4100) works on the principle similar to microscopic analyses: particles in the suspension are photographed and analyzed automatically by the image analysis system (Brightwell Technologies Ltd. 2007). Both particle counters could measure particles down to an equivalent circular diameter of 1 μm.

Results and Discussion

Table 3 shows the treated water quality and sizes of particles obtained in this study. For comparison, the results obtained in the earlier study by Gorczyca and Zhang (2007) are also shown in Table 3.

Figure 6 shows alum coagulation floc size distributions as obtained with the Spectrex particle counter. Figure 7 shows a typical floc size distribution as obtained with the Brightwell Dynamic particle counter. The average floc sizes obtained with the microscope and the Brightwell Technologies counter were reasonably close, within 0.5 to 2.2 μm. This indicates that the Brightwell particle counter can be used to determine distributions of flocs larger than 1 μm.

Unlike the microscopic analyses, the determinations with particle counters are quick and do not require sample preparation, and, therefore, the particles in DAF effluent were measured using the particle counters only. The highest difference in the average particle size as measured using the particle counter and the microscopes was found for raw (tap) water. That could be due to the fact that many particles in the tap water were algal filaments. The particle counter will report different sizes of such elongated particles depending on the particle orientation. Therefore, the results obtained for tap water presented in this paper are not used in any further analyses and comparisons.

The DAF effluent colour and turbidity after coagulation with 25, 40, or 60 mg/L of alum was very similar. After 60 mg/L of alum, the concentration and size of particles in DAF effluent were the smallest (Table 3). This indicates that the particle removal was best at 60 mg/L. Therefore, the dose of 60 mg/L of alum was considered optimum for solid/liquid separation in the DAF unit in this study. At this dosage, the mean size of coagulation flocs was found to be about 30 μm.
Comparison of the Results of this Study with the Earlier Study

Several novel microscopic floc sizing procedures have been introduced in this paper as compared with the earlier study conducted by Gorczyca and Zhang (2007). The stereoscopic microscope (Nikon SMZ 800) used for floc sizing in this study allowed for elimination of agar which improved detection of flocs smaller than 5 μm.

Application of the particle counter further improved detection of small particles down to an equivalent diameter of 1 μm. Particle counters used in this study also allowed for rapid measurements of the particles in the DAF treated effluent and provided information on particle removal efficiency. In this study, significantly

![Fig. 6. Alum floc size distributions at different coagulant dosages as obtained with Spectrex PS-2200 particle counter.](image)

![Fig. 7. Typical alum floc size distribution as obtained with Brightwell Technologies Dynamic particle counter (DPA 4100).](image)
larger numbers of flocs have been measured as the random floc sampling was conducted based on the statistical design.

As a result of these significant improvements to the operation of the DAF unit and particle sizing procedures, a dosage of 60 mg/L of alum was considered optimum as compared with 25 mg/L selected in the earlier paper (Gorczyca and Zhang 2007).

Floc to Bubble Size Relation

The sizes of air bubbles were not measured in this study. Assuming the average size of the bubbles of 40 μm (Edzwald 1995, 2007), the average sizes of flocs was smaller but close to the size of the air bubbles produced by the saturator. Unfortunately, the estimates provided by Edzwald are not based on measurement of bubble sizes; these numbers simply provide some guidelines as to the bubble size range.

Han et al. (2002) actually measured bubble size, and provided distribution of these sizes. The reported distribution of sizes of bubbles at the saturator pressure of 550 kPa in DAF at the saturator pressure of is lognormal, with the mean size around 30 μm, which is smaller than the average bubble size suggested by Edzwald (2007).

In this study, the mean floc size at the optimum dosage of 60 mg/L was about 30 μm and was similar to the mean size of the air bubble measured by Han et al. (2002). Similar size of floc and bubble indicates that flocs act predominantly as nuclei for bubble formation.

Comparison of Bench- and Pilot-Scale DAF Studies

There were many differences between the bench- and pilot-scale DAF studies besides those listed in Table 1. The pilot study influent water was taken directly from Deacon Reservoir and contained less chlorine than the Winnipeg tap water. Additional chlorine content in the water may have reduced the coagulant demand in the bench-scale study. Coagulant aid and pH adjustment were applied in the pilot-scale but not in the bench-scale study described in this paper. In addition, the selection of optimum dose in the pilot study was based not only on turbidity and colour, but also on the removal of the TOC. TOC was not analyzed in this study due to the lack of laboratory equipment. Despite many differences between the DAF pilot- and the bench-scale study described in this paper, the same optimum coagulant dosages were found in both cases.

Conclusions

Bench-scale DAF experiments were conducted to study optimal floc size distribution for separation in flotation. Tap water in the City of Winnipeg, Manitoba, (Canada) was used in the tests. The following conclusions were made:

1. The average floc sizes obtained with the microscope and the Brightwell Technologies counter were reasonably close, within 0.5 to 2.2 μm. This indicates that the Brightwell particle counter can be used to determine distributions of flocs larger than 1 μm. Unlike the microscopic analyses, the determinations with particle counters are quick and do not require sample preparation.

2. Analyses of particles in the DAF effluent indicated that at the coagulation with 60 mg/L of alum, the removal of particles was best; therefore, this coagulant dosage was considered optimal. The average size of floc at the dose of 60 mg/L of alum was about 30 μm. This is similar to the size of the bubbles measured at the pressure of 530 kPa reported in the literature.

3. Predominantly similar size of floc and bubble indicates that during the optimal DAF operation, flocs act as nuclei for bubble formation.

Acknowledgments

This work was supported by National Science and Engineering Research Council of Canada. George Zhang was involved in experimental work presented in this paper. We also thank the reviewers for helpful comments on an earlier version of the manuscript.

References


Received: 27 August 2007; accepted: 28 May 2008.
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