The Effect of Short-Term Dissolved Oxygen Transients on Activated Sludge

Yi Zhang and D. Grant Allen*

Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, Ontario, Canada M5S 3E5

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 air aeration (DO < 1 mg/L). After air 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 (FrØlund et al. 1996; Durmaz and Sanin 2001). Depending on the origin of wastewater, humic substances may also be a main EPS component. Flemming 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 flocc 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 flocc 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; Sobeck and Higgins 2002). It is known that divalent cations (e.g., Ca²⁺ and Mg²⁺) 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 m³/day of wastewater is treated by the plant. During the sampling period, the average BOD₅, 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 and purging was recorded as time 0. The overall reaction time was 10 hours. The transient reactors were continuously purged with N₂ 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 (Wilen 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; FrØlund 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⁺, Ca²⁺, and Mg²⁺), 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) (FrØlund 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 Ca²⁺ under the DO transient condition. One milliliter of soluble biopolymers solution was acidified with 1 mL of concentrated HNO₃ 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 N₂ 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 N₂ purging and turning on aeration.

A low DO concentration caused a significant increase in the supernatant turbidity (Fig. 1). After 2 hours of N₂ 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 N₂ 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 x 10⁻²). The error bars are +/- one standard deviation of turbidity measurements.

Fig. 2. Microscopic observation of supernatant: (a) control; (b) transient. The images were taken by Axiovert 200, inverted microscope. The bar represents 50 μm.
control samples remained low and stable (around zero). In contrast, soluble proteins in transient samples increased under the low DO. After 6 hours of \( \text{N}_2 \) 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 \( Ca^{2+} \) 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 \( Ca^{2+} \), and 18 mg/L of \( Mg^{2+} \) in bulk phase. During 6-hour DO limitation, the bulk liquid contained more \( K^+ \) (40% higher) and less \( Ca^{2+} \) (20 to 30% lower). When the DO concentration was restored in the last 4 hours, the concentrations of bulk \( K^+ \) and \( Ca^{2+} \) returned close to those in control samples, suggesting reversible changes in the levels of \( K^+ \) and \( Ca^{2+} \) under the DO transient. In comparison, no significant changes in \( Na^+ \) and \( Mg^{2+} \) were observed under DO limitation.

A negligible amount of extracellular bulk \( Ca^{2+} \) 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 \( Ca^{2+} \) level occurred before and after the acidification, a negligible amount of \( Ca^{2+} \) was recombined with soluble biopolymers under DO limitation. In other words, the decreasing levels of bulk \( Ca^{2+} \) 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
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Fig. 6. Changes in soluble polysaccharides under low DO: No significant difference between the control and transient samples from $t = 2$ to 6 hours ($p = 0.43$). The error bars represent +/- one standard deviation of bulk polysaccharides.

Fig. 7. Changes of bulk $K^+$ under DO transient: From $t = 2$ to 6 hours, transient samples had a higher concentration of $K^+$ than control samples ($p = 1.6 \times 10^{-3}$). The error bars are +/- one standard deviation of bulk $[K^+]$ measurements.

Fig. 8. Changes of bulk $Ca^{2+}$ under DO transient: From $t = 2$ to 6 hours, transient samples had a lower concentration of $Ca^{2+}$ than control samples ($p = 1.7 \times 10^{-2}$). The error bars are +/- one standard deviation of bulk $[Ca^{2+}]$ measurements.

Fig. 9. Changes of $Ca^{2+}$ under acidification: There was no significant difference between the samples before and after acidification ($p = 0.59$). The error bars are +/- one standard deviation of $[Ca^{2+}]$ measurements.

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.

Fig. 10. Turbidity changes in enzyme tests: Operating temperatures for trypsin and cellulase were 37ºC, for amylase was 25ºC. Two reference samples were prepared at 25ºC (C1) and 37ºC (C2), respectively. The error bars represent +/- one standard deviation of turbidity measurements.
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 biofloc 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 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⁺).

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