

# Structure–function dynamics and modeling analysis of the micro-environment of activated sludge floc

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**Abstract** Biodegradation by microorganisms and mass transfer resistance in the micro-environment of activated sludge floc can cause changes in substrate and dissolved oxygen concentrations within the floc and can contribute to stratification of microbial processes inside the flocs. In this study, an integrated model of the microenvironment of the activated sludge floc was developed for floc from wastewaters from several sources and of varying strengths for dynamic simulation of the combined biological processes of COD and nitrogen removal. The model simulation results and measured profiles show the heterogeneous and gradient-governed microenvironment of activated sludge floc under different substrate and bulk oxygen concentrations. The substrate concentration increase zones inside the floc were present in all activated sludge floc from the Miller Brewing Co. wastewater treatment facility (high pollutant strength), with an oxygen penetration depth of only 0.15 mm into the outer layer. The anoxic and substrate concentration increase zones also dominated in the activated sludge floc from the Mill Creek Plant influent (medium pollutant strength), with the outer layer (0.20 mm) participating in the metabolism of the pollutants. The radius of the substrate concentration increase zone inside the sludge floc decreased with pollutant removal along the length of the tank. When the pollutant concentration in the bulk wastewater was low (Muddy Creek Plant), the substrate concentration increase zone disappeared; the whole floc was aerobic and in a high redox status. Our experiments and model analyses demonstrate that the microorganisms' structure-functions inside activated sludge floc change with the bulk substrate concentration and dissolved oxygen concentration.

**Keywords** Activated sludge floc; dissolved oxygen; mathematical modeling; microelectrode; microenvironment; nutrient removal; redox potential (ORP)

## Introduction

The activated sludge process uses mixed populations of bacteria to treat a variety of wastewaters. The microbial activity of activated sludge floc has a significant effect on pollutant removal. Although many efforts have been aimed at process engineering and a series of mathematical models for the activated sludge process has been developed and applied for both process control and optimal operation (Chambers, 1982; Dold, 1990; Hao *et al.*, 1983; Palm *et al.*, 1980; Wentzel *et al.*, 1992; Henze *et al.*, 2000), our current knowledge of the activated sludge floc microbial community and structure–function correlation, and consequently a microbiological understanding of the activated sludge process itself, is very limited. A detailed model analysis of activated sludge flocs is necessary (Dullstein and Rabiger, 2000). Until now, a direct validation for the spatial stratification and modeling inside activated sludge flocs under different wastewater conditions has not been reported. For the first time, the microbial processes, dissolved oxygen (DO) and the redox (ORP) status inside sludge flocs in different DO and COD concentrated wastewaters were investigated in this study by combining integrated modeling and microelectrode techniques. The proposed model takes into consideration the mass transfer of substrate and oxygen, the biological reactions, nitrification, the biomass hydrolysis and the endogenous respiration inside activated sludge flocs. The coefficients were set up according to the ASM3 model (Henze *et al.*, 2000) and the data acquired from the experiments on full-scale aeration tanks.

The model validation was carried out with the microelectrode profiles of the activated sludge floc.

## Materials and methods

### Chemical profile measurements of activated sludge floc

Two microelectrodes (DO and ORP), with tip diameters of 3–15  $\mu\text{m}$ , were used to investigate the spatial stratification of microbial processes inside activated sludge floc. Microelectrodes have been developed and used in our lab for the last decade (Fu *et al.*, 1994; Zhang and Bishop, 1994; Zhang *et al.*, 1994; Zhang and Bishop, 1995; Yu and Bishop, 1998; Li and Bishop, 2002). The microelectrodes described below were used as working electrodes. An Ag/AgCl millielectrode (Microelectrode Co., no. MI 401) was used as the reference electrode throughout this study. An up-flow chamber was used to keep the floc particles suspended in the flowing liquid, but stationary, while the microelectrode penetrated through the floc. The reference electrode and microelectrode were used in the flowing solution inside the up-flow chamber (Li and Bishop, 2002).

The microprofile measurements were carried out in a Faraday Cage. After the activated sludge floc was observed to be in suspension just above the nylon net in the chamber, a microelectrode was located 1.0 mm distance from the surface of the floc particle. The position of the microelectrode was controlled using a micro-manipulator. A Nikon stereomicroscope, CCD camera and color monitor were used to monitor the location of the microelectrode during all measurements. The microelectrode was moved into the floc particle, and readings were recorded at 100  $\mu\text{m}$  intervals.

### Mathematical model

The proposed model is based on the ASM3 model (Henze *et al.*, 2000) and the data acquired from experiments using full-scale aeration tanks. Matlab was used for model simulation. The concentrations of substrate and oxygen were calculated inside the floc along the aeration tanks' lengths.

$$\frac{\partial S}{\partial t} = \frac{1}{r^2} \frac{(D_s \cdot r^2 \frac{\partial S}{\partial r})}{\partial r} - \frac{\mu_{\max}}{Y_s} \cdot \frac{S}{S + K_s} \cdot \frac{O}{K_o + O} \cdot X + K_{lys} \cdot X - K_{res} \cdot \frac{S}{S + K_s'} \cdot \frac{O}{K_{od} + O} \cdot X \quad (1)$$

$$\frac{\partial O}{\partial t} = \frac{1}{r^2} \frac{(D_o \cdot r^2 \frac{\partial O}{\partial r})}{\partial r} - \frac{\mu_{\max}}{Y_o} \cdot \frac{S}{S + K_s} \cdot \frac{O}{K_o + O} \cdot X - \alpha \cdot K_{res} \cdot \frac{S}{S + K_s'} \cdot \frac{O}{K_{od} + O} \cdot X - \frac{\mu_{\max NO}}{Y_{oNO}} \cdot \frac{NH}{NH + K_{sNH}} \cdot \frac{O}{K_o + O} \cdot X_a \quad (2)$$

In order to develop the mathematical equations, the following assumptions for activated sludge flocs were made:

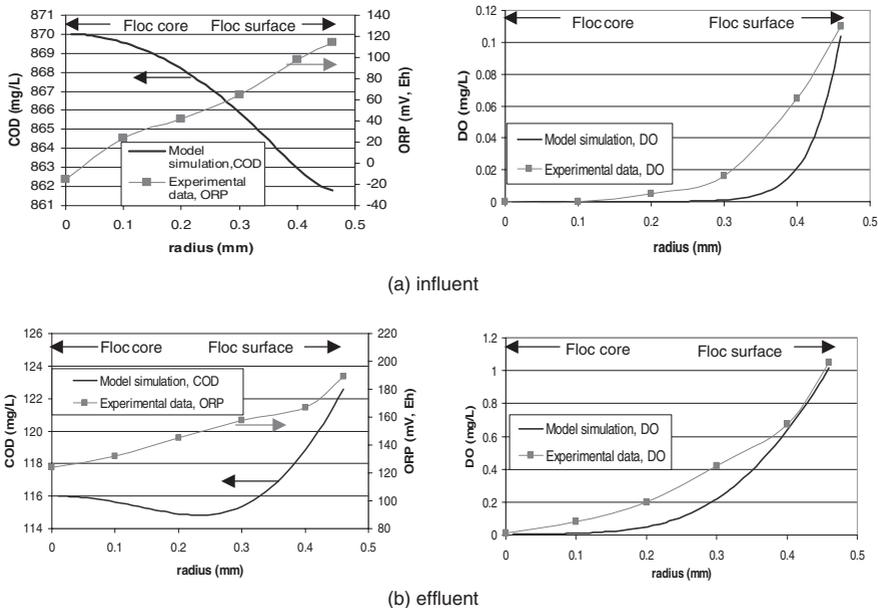
1. The activated sludge floc particle is spherical in shape and symmetry, although during the micro-profile measurements, the floc particles were observed to be not exactly spherical.
2. Although the species of microorganisms change spatially inside activated sludge floc, which leads to the heterogeneity found inside floc particles, the cell density is assumed uniform throughout the floc radius.
3. Molecular diffusion controls the transfer of the nutrients into the floc particle, and the diffusion coefficient of each is assumed constant throughout the floc matrix (Abbassi *et al.*, 2000).

4. According to ASM3 (Henze *et al.*, 2000), the metabolisms (cell growth, endogenous respiration) of heterotrophic organisms and autotrophic nitrifiers are clearly separated. In model simulations, the proportion of nitrifiers ( $X_a$ ) and the heterotrophic organisms ( $X$ ) in activated sludge floc varied with different wastewater treatment processes. The percentage of nitrifiers was assumed to be 10% in the Muddy Creek Plant activated sludge (full nitrification), and less than 2% in the Mill Creek Plant activated sludge (minimal nitrification) (Seviour and Blackall, 1999).

## Results and discussion

### Modeling and microprofiles of activated sludge flocs – Miller Brewing Co. wastewater treatment facility

According to the model calculations and microelectrode-measured profiles, the micro-environment of the activated sludge floc is gradient governed, resulting in the mass diffusion limitation inside the floc. The profiles clearly show the heterogeneity in the activated sludge floc. The influent of the Miller Brewing Co. wastewater treatment facility contained 862 mg/L COD, with a DO concentration of 1.5 mg/L. DO profiles showed that DO was depleted at 0.15 mm into the outer layer of the activated sludge floc, indicating a high oxygen consumption rate by the microorganisms due to the high organic contaminant concentration. The redox potential dropped from 120 mV (Eh) on the activated sludge surface to  $-20$  mV at the sludge core (Figure 1a). Moreover, the simulated COD profile showed the substrate concentration increased in the whole sludge floc in the direction of the floc core. There are perhaps three reasons for this substrate concentration increase in the anoxic zone: (1) Cell hydrolysis. In the anoxic zone, with sufficient substrate, hydrolysis of cells and extracellular polymeric material (EPS) may occur (Abbassi *et al.*, 2000). New soluble substrate is generated by the cell lysis. (2) The storage and accumulation process. Some researchers have suggested the aerobic/anoxic storage of readily biodegradable substrate by heterotrophic organisms (Gujer *et al.*, 1999). The stored particulate substrate is



**Figure 1** The model-based and micro-electrode experimental profiles of substrate, oxygen and ORP in activated sludge floc from aeration tanks at the Miller Brewer Co. Wastewater Treatment Facility

(a) Influent: (bulk COD: 862 mg/L, bulk DO: 1.5 mg/L)

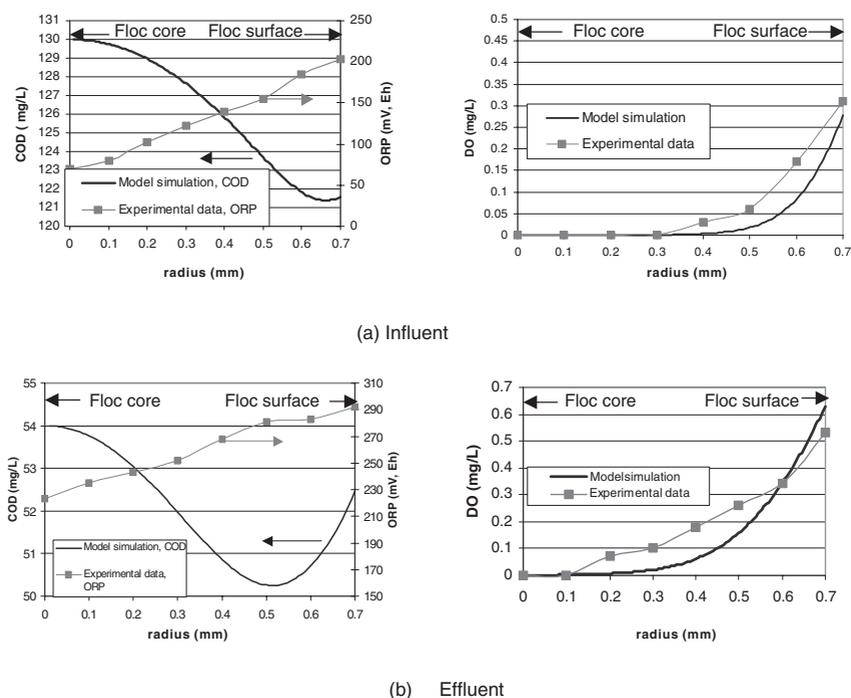
(b) Effluent: (bulk COD: 123 mg/L, bulk DO: 2.3 mg/L)

then transported into the biomass, maintained in storage there and later transformed into low molecular weight soluble metabolic intermediates. These soluble metabolites or solubilized extracellular polymers may be released. (3) Diffusion resistance for the substrate. The soluble metabolites created in the floc core diffuse toward the bulk solution, but their mass transfer rate is limited. Therefore, the substrate concentration in the floc center may be higher than that in the bulk solution or in the actively degrading zone of the floc, which was caused by the mass transfer resistance for substrate, the cell hydrolysis process (Takacs and Fleit, 1995) and the storage and accumulation process.

COD in the effluent dropped to 123 mg/L, with a DO concentration of 2.3 mg/L. Oxygen penetrated deeper inside the activated sludge floc compared with the influent sludge. Along with biodegradation and DO penetration, redox potential (ORP) in the sludge core increased to 118 mV. The COD profile clearly showed the outer layer (0.20 mm) of the sludge floc took part in the metabolism of contaminants, and the substrate increase zone was limited in the sludge core; it was much less significant than in the influent floc (Figure 1b). The differences in COD, ORP and DO profiles between the influent and effluent activated sludge flocs indicate the microbial processes inside activated sludge floc changed along with the bulk wastewater quality.

#### Modeling and microprofiles of activated sludge flocs – Mill Creek municipal wastewater treatment facility

In the Mill Creek plant aeration tank influent (bulk COD: 124 mg/L, bulk DO: 0.6 mg/L, minimal nitrification), the exterior part of the floc (upper 0.10 mm) was able to participate in pollutant metabolism, while the anoxic interior of the floc exhibited rapidly decreasing effectiveness (Figure 2a). The simulation substrate concentration (COD) is lower in the



**Figure 2** Model-based and micro-electrode experimental profiles of substrate, oxygen and ORP in activated sludge floc from aeration tanks at the Mill Creek Plant

(a) Influent: (bulk COD: 124 mg/L, bulk DO: 0.61 mg/L, bulk  $\text{NH}_4$ : 15.3 mg/L, bulk  $\text{NO}_3$ : 0.1 mg/L)

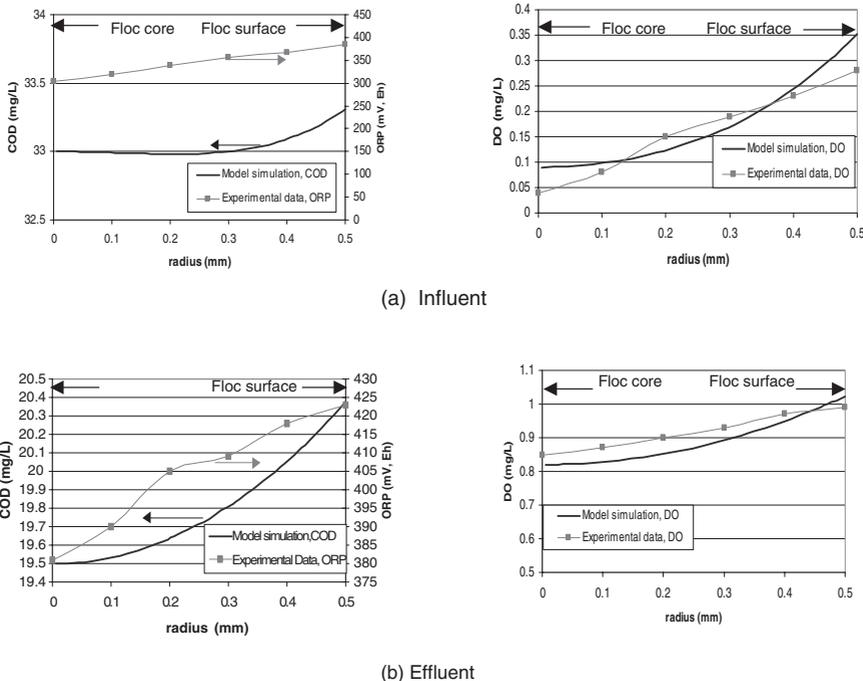
(b) Effluent: (bulk COD: 55 mg/L, bulk DO: 1.0 mg/L, bulk  $\text{NH}_4$ : 15.52 mg/L, bulk  $\text{NO}_3$ : 0.1 mg/L)

outer floc layer, but increased in the anoxic zone in the direction of the floc core, in some cases exceeding the COD in the bulk liquid, similar to that in activated sludge floc from the Miller Brewing Co. wastewater treatment facility. Oxygen-limiting conditions were considered in the substrate biodegradation. Because of the substrate concentration increase and the oxygen depletion, the ORP decreased inside the floc, dropping to 50 mV (Eh) in the core of the floc.

In the aeration tank effluent activated sludge floc (bulk COD: 54 mg/L, bulk DO: 1.1 mg/L), the model indicates that the substrate concentration increase zone should still exist in the activated sludge core (Figure 2b), but the concentration increase zone should be smaller than in the aeration tank influent. Efficient substrate biodegradation occurred in the outer 0.22 mm of the sludge floc, deeper than that in the sludge floc from the influent. DO penetrated deeper inside the sludge flocs. The aerobic zone was enlarged to the outer 0.43 mm. ORP increased to 332 mV in the bulk effluent and 220–290 mV inside the floc particle.

### Modeling and microprofiles of activated sludge flocs – Muddy Creek municipal wastewater treatment facility

The contaminant concentration in the Muddy Creek Wastewater Treatment Facility was the lowest among the three tested plants. This led to a lower oxygen consumption rate by microorganisms inside the activated sludge floc. In the Muddy Creek Plant aeration tank influent (bulk COD: 34 mg/L, bulk DO: 0.54 mg/L, full nitrification), the anoxic zone was limited to only 0.1 mm radius at the activated sludge floc core, according to both the model and actual measurements (Figure 3a). The decrease in DO concentration inside the floc was low, as a result of the lower oxygen utilization rate. No substrate concentration increase at the floc core was observed in the substrate concentration simulation curve, indicating that



**Figure 3** Model-based and micro-electrode experimental profiles of substrate, oxygen and ORP in activated sludge floc from aeration tanks at the Muddy Creek Plant

(a) Influent: (bulk COD: 34 mg/L, bulk DO: 0.5 mg/L, bulk  $\text{NH}_4^+$ : 5.2 mg/L, bulk  $\text{NO}_3^-$ : 4.9 mg/L)

(b) Effluent: (bulk COD: 21 mg/L, bulk DO: 1.13 mg/L, bulk  $\text{NH}_4^+$ : 0.89 mg/L, bulk  $\text{NO}_3^-$ : 18.1 mg/L)

mass transfer resistance was low and biodegradation was almost finished in the aerobic zone. The ORP of the floc core was 300 mV, much higher than that in the Mill Creek plant floc, as a result of the lower substrate concentration and deeper oxygen penetration.

In the Muddy Creek aeration tank effluent (bulk COD: 22 mg/L, bulk DO: 1.1 mg/L), the entire sludge floc particle was aerobic (Figure 3b). Both simulated and measured oxygen profiles show that DO was still at 0.8 mg/L in the floc core. With sufficient oxygen inside the sludge floc, aerobic respiration (which led to biomass growth and substrate loss) overcame the substrate accumulation. The entire floc particle was active in aerobic substrate biodegradation, and substrate concentrations kept dropping to the floc center.

## Conclusions

The combination of the proposed model (based on ASM3) and the microelectrode measurement data provide a better understanding of the complex, detailed ecological micro-system of activated sludge flocs. The results validate the heterogeneity of the activated sludge floc. Oxygen is a limit factor in substrate degradation in the gradient-governed micro-environment. Dissolved oxygen is necessary for reduction of the substrate inside the sludge floc. The model simulations and the experimental microelectrode profiles indicate the microenvironment of activated sludge floc; with the outer layer efficiently participating in the metabolism of the pollutants, while the anoxic interior of the floc rapidly decreases in activity. The model predicts that cell hydrolysis, aerobic/anoxic substrate storage and diffusion resistance inside the sludge floc lead to the substrate concentration increase zone inside the floc, in which the rate of substrate biodegradation would be exceeded by the rate of substrate formation. With pollutants removed from the system, oxygen could penetrate deeper into the floc, resulting in a decrease of the substrate concentration increase zone and the enlargement of the aerobic region. It is also shown that free-living microorganisms in the boundary layer outside the sludge floc participated in the substrate removal and caused some of the oxygen consumption.

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