



## Micro-environment characteristics and microbial communities in activated sludge flocs of different particle size

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### HIGHLIGHTS

- ▶ Microelectrodes and molecular culture-independent techniques were applied.
- ▶ The micro-environment and bacteria in flocs with different size were detected.
- ▶ The DO,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentration were distinct in flocs of different size.
- ▶ The bacterial population was closely related to the micro-environment in flocs.

### ARTICLE INFO

#### Article history:

Received 6 May 2012

Received in revised form 27 July 2012

Accepted 2 August 2012

Available online 9 August 2012

#### Keywords:

Micro-environment

Microbial community

Microelectrodes

Fluorescence *in situ* hybridization

Activated sludge flocs

### ABSTRACT

Microorganisms in activated sludge flocs (ASF) play important roles in the wastewater treatment process. However, the interplay between micro-environmental variation and microbial responses within ASF is poorly understood. In this study, microelectrodes and molecular culture-independent techniques were applied to detect the internal environment, microbial composition, and distribution in flocs with different particle size. Results showed dissolved oxygen (DO) concentrations within the center of the flocs were less than at the surface, and the DO concentration distributions were distinct in flocs of different particle size. With floc particle size increasing from 100 to 250  $\mu\text{m}$ , the DO concentrations in the floc centers decreased 10–55%, respectively, while no distinct change was observed in flocs of less than 100  $\mu\text{m}$ . Similar phenomenon occurred for the distributions of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in flocs. Microbial structure indicated bacterial compositions and distributions were heterogeneous and responded to micro-environment variation in flocs of different particle size.

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### 1. Introduction

Activated sludge treatment of wastewater is one of the most important biotechnological processes in a wastewater treatment plant (WWTP). Generally, activated sludge flocs (ASF) are in the form of aggregates of microorganisms, suspended solids, and extracellular polymers (Jin et al., 2004). Removal of pollutants from wastewater mainly depends on the microbial composition and activity of activated sludge (Nadarajah et al., 2007; Pholchan et al., 2010; Wells et al., 2011). Thus flocs as microbiological units play an essential role in the operation of WWTPs. Better understanding the characteristics of inter-floc bacterial communities will help clarify the processes of pollutant biodegradation.

Previous research has shown that bacterial distribution in flocs is heterogeneous. For example, the distribution of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) was

observed to be stratified in activated sludge by fluorescence *in situ* hybridization (FISH) (Schramm et al., 1999). Efforts have been made to link the relationship between the structure and function of microbial systems inside activated sludge flocs by using mathematical modeling, which show that microbial distribution is related to their resident environment inside activated sludge (Martins et al., 2004).

In addition to the model simulation, the ASF micro-environment has also been directly investigated. With the rapid development of microelectrode techniques in recent years, it was now possible to detect the inner characteristics of micro spaces, such as biofilms and granules (Yu and Bishop, 2001; Li and Bishop, 2002; Chu and Lee, 2004; Kishida et al., 2006; Shanahan and Semmens, 2006). Microelectrodes had been applied to detect the micro-environment in activated sludge aggregates. The pH, oxidation–reduction potential (ORP), DO,  $\text{NH}_4^+$ , and  $\text{NO}_3^-$  concentrations were shown to decrease from the surface to the center of activated sludge aggregates with particle size of more than 1000  $\mu\text{m}$  (Li and Bishop, 2003, 2004; Satoh et al., 2003; Wang et al., 2011). Analysis of the DO diffusion in a single floc with 600  $\mu\text{m}$  sized particles was conducted

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