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Microbial responses to membrane cleaning using sodium hypochlorite in membrane bioreactors: Cell integrity, key enzymes and intracellular reactive oxygen species

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ABSTRACT

Sodium hypochlorite (NaClO) is a commonly used reagent for membrane cleaning in membrane bioreactors (MBRs), while it, being a kind of disinfectant (oxidant), may impair viability of microbes or even totally inactivate them upon its diffusion into mixed liquor during membrane cleaning. In this study, we systematically examine the effects of NaClO on microorganisms in terms of microbial cell integrity, metabolism behaviours (key enzymes), and intracellular reactive oxygen species (ROS) under various NaClO concentrations. Different proportions of microbial cells in activated sludge were damaged within several minutes dependent on NaClO dosages (5–50 mg/g-SS), and correspondingly organic matters were released to bulk solution. Inhibition of key enzymes involved in organic matter biodegradation, nitrification and denitrification was observed in the presence of NaClO above 1 mg/g-SS, and thus organic matter and nitrogen removal efficiencies were decreased. It was also demonstrated that intracellular ROS production was increased with the NaClO dosage higher than 1 mg/g-SS, which likely induced further damage to microbial cells.

1. Introduction

In recent decades, membrane bioreactor (MBR) technology has been increasingly used worldwide for wastewater treatment due to its high-quality effluent, reduced footprint and decreased sludge yield compared to conventional activated sludge systems (Judd, 2008). However, membrane fouling, which is caused by the deposition of sludge flocs, extracellular polymeric substances (EPS) and soluble microbial products (SMP) (Lin et al., 2009, 2014) on membrane surface or adsorption within the internal structure of membranes, results in flux reduction or trans-membrane pressure increase. In order to remove tenacious membrane foulants, chemical cleaning of membranes is an essential part of operation in MBRs, and several protocols including chemically enhanced backflush (CEB), maintenance cleaning [also termed cleaning-in-place (CIP)], recovery cleaning and *ex-situ* cleaning are generally adopted (Wang et al., 2014a). However, during CEB and CIP, chemical reagent is dosed into the backwashing water, which may diffuse and/or permeate through membrane into mixed liquor. Moreover, CEB and CIP are carried out on a daily/weekly and monthly basis, respectively. Therefore, microorganisms are frequently exposed to chemical reagents in MBRs.

Sodium hypochlorite (NaClO) is a commonly used cleaning reagent in CEB and CIP (Judd and Judd, 2012; Wei et al., 2011; Wang et al., 2014b) in MBRs due to its chemical stability, good cleaning efficiency against organic foulants and easy combination with other cleaning detergents (Dukan and Touati, 1996; Ono et al., 2012; Ramos et al., 2014; Virto et al., 2005). A large number of publications have focused on NaClO dosages, cleaning mode, duration and cleaning efficiencies in MBRs (Puspitasari et al., 2010; Piasecka et al., 2015; Wang et al., 2014a), and also their impacts on the integrity of membrane materials (Abdullah and Bérubé, 2013; Hanafi et al., 2014; Wang et al., 2010). However, studies on the effects of NaClO on activated sludge during membrane cleaning in MBRs are limited. Available literature shows that the nitrification

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rate was impaired by 69% within 30 min in the presence of 5 mg/g-suspended solids (SS) (Lee et al., 2013), and further increase of NaClO dosage resulted in deterioration of organic matter degradation (Lim et al., 2005). Wang et al. (2014a) reported that intracellular substances were released from sludge flocs when the ClO^- dosage was about 21 mg/g-SS with reaction time of 3 h. Nevertheless, there is a lack of a systematic study on the interaction mechanisms between sodium hypochlorite and activated sludge for membrane cleaning in MBRs.

It is known that cell membrane consists of a lipid bilayer. The C═C double bonds of lipid bilayers are susceptible to the attack of strong oxidizing agents and can be fragmented by hypochlorite ions (Fukuzaki, 2006). This may change cell membrane's permeability of microorganisms and consequently impact their metabolism behaviours. During aerobic biodegradation of organic matters, the basic biochemical processes in microbial cells are tricarboxylic acid (TCA) cycle and oxidative phosphorylation (Rittmann and McCarty, 2002). Therefore, the performance of organic matter removal is related to the activity of dehydrogenase (DHA) which is involved in TCA cycle, and also related to the quantity of adenosine triphosphate (ATP) which is mostly generated during oxidative phosphorylation. In nitrogen transformation, the key enzymes are ammonia monooxygenase (AMO) and nitrite oxidoreductase (NOR) for nitrification, and nitrate reductase (NAR) and nitrite reductase (NIR) for denitrification (Zheng et al., 2011). It can be inferred that the presence of NaClO may disrupt cell membrane and then affect the enzyme activities and ATP production, thus inhibiting the removal efficiency of organic matter and nitrogen. However, these have not been systematically studied in MBRs under relevant NaClO dosages for membrane cleaning.

The objective of this study is therefore to systematically investigate the effects of NaClO on activated sludge during membrane cleaning in MBRs. The influential mechanisms are discussed through monitoring microbial cell integrity, metabolism behaviours (key enzymes), and intracellular reactive oxygen species (ROS) under various NaClO concentrations. Flow cytometry (FCM) combined with fluorescent dye staining was used to determine the cell integrity of microbes. DHA activity and ATP quantity were analyzed under different NaClO dosages to reflect the heterotrophic metabolism behaviours, while activities of key enzymes related to nitrification and denitrification were measured to indicate nitrogen remove capability. Intracellular ROS was also determined to highlight the oxidative stress in microbial cells in the presence of NaClO. This study provides information on microbial responses to membrane chemical cleaning that will support to establish a more comprehensive understanding of chemical cleaning for MBRs.

2. Materials and methods

2.1. Sludge samples and chemicals

In this study, activated sludge was taken from a lab-scale anoxic/oxic (A/O) MBR fed with municipal wastewater, which was located in the Quyang Municipal Wastewater Treatment Plant, Shanghai, China. The reactor had an anoxic zone with effective volume of 22 L and an oxic zone with effective volume of 30 L. Four flat-sheet membranes (SHZZ-MF, Zizheng Environment Inc., Shanghai, China) with a mean pore size of 0.2 μm were installed in the oxic zone. The membranes were made of polyvinylidene fluoride (PVDF) with polyester non-woven fabric as supporting material. The total effective filtration area was 0.63 m^2. These membranes were connected to a peristaltic pump for extracting effluent. An air pump was used to provide oxygen for microorganisms and to induce a cross-flow velocity along membrane surface. Membrane flux was maintained at about 15 L/(m^2 h). Total hydraulic retention time (HRT) was 6.6 h. Sludge retention time (SRT) was 60 days via extracting excess sludge daily.

Unless specified otherwise, all chemicals were of analytical grade with purity over 99%. NaClO (~6%, reagent grade), sodium sulfite, ammonium chloride, sodium nitrate, sodium nitrite and sodium chloride were purchased from Aladdin (China). The buffer, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (10 mM, pH = 7), was also obtained from Aladdin (China). Distilled water was used in all preparations and experiments.

2.2. Experimental design

A series of concentrations (0, 1, 5, 10, 20, 50 mg/g-SS) of NaClO were adopted for this study, which were chosen based on chemical cleaning protocols used in previous studies of MBRs (Judd and Judd, 2012; Wang et al., 2014a). The details of calculation process are documented in the Supporting Information (SI). These concentrations were the dosages that activated sludge was potentially exposed to in MBRs. In real applications, cleaning condition might not always be the same as these cases. However, attention should be paid to avoid inappropriate cleaning protocols which might negatively affect MBR performance. Prior to the experiment, activated sludge samples collected were washed and centrifuged twice (4000 × g, 10 min) to remove supernatant. In order to keep the same operating conditions in each test, the suspended solids (SS) concentration was then adjusted to 10 g/L, a typical sludge concentration in practical MBRs. After that, 300 mL of the bulk solution was poured into a jacketed glass vessel that was magnetically stirred. In order to avoid the influence of ionic strength on microorganisms, appropriate amount of NaCl was added to adjust the ionic strength to 15.4 mM for all the vessels. Since the duration of most CIP and CEB ranged from several minutes to 4 h (Huang and Wen, 2012; Judd and Judd, 2012; Wang et al., 2014a, b), the longest exposure duration was thus set as 4 h. Followed by NaClO injection with pre-determined concentrations, samples of the bulk solution were periodically collected during 4 h exposure from the vessels for further analysis. After 4-h exposure to NaClO, the bulk solution was centrifuged at 4000 × g for 10 min to discard supernatant. The remaining pellet was washed twice to remove residual sodium hypochlorite and re-suspended with NaCl solution. Organic matter biodegradation, nitrification and denitrification rate were then determined using these sludge samples, respectively.

2.3. Analytical methods

2.3.1. Organic matter release

Organic matter release was evaluated through monitoring the changes of total organic carbon (TOC) concentration in supernatant during 4-h exposure. Sludge samples were collected and appropriate amount of sodium sulfite, as a quenching agent of the residual NaClO, was dosed immediately. The samples were then subject to centrifugation (8000 × g, 5 min) and supernatant was collected and filtered by a membrane (0.45 μm, PTFE, Anpel). The TOC concentration in the filtrate was then measured using a TOC analyzer (TOC-L_{CPH}, Shimadzu Co., Japan).

2.3.2. Cell integrity

Double staining with calcein-AM (CAM) and propidium iodide (PI) was used to assess cell membrane integrity by FCM based on Hiraoka and Kimbara's method (2002). Briefly, sludge samples were harvested by centrifugation (8000 × g, 5 min). After discarding supernatant, the remaining pellet was washed by HEPES buffer (10 mM, pH = 7.0). Then the pellet was re-suspended and diluted for 100 times in 15.4 mM NaCl solution. Ultrasonication with a power density of 1.3 W per milliliter of mixture was applied