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Effect of quorum quenching on biofouling and ammonia removal in membrane bioreactor under stressful conditions

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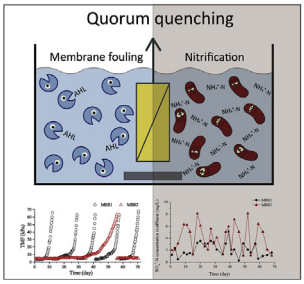
HIGHLIGHTS

- Effect of QQ on ammonia removal in MBR under stressful conditions was investigated.
- Ammonia removal was compromised by QQ in MBR operated under short HRT.
- QQ made nitrification more susceptible to inhibitors and low temperature.

GRAPHICAL ABSTRACT



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ABSTRACT

Quorum quenching (QQ) has been used to control biofouling in membrane bioreactors (MBRs), but the effect of QQ on the performance of MBR has not been systematically studied. This study investigated the effect of QQ on ammonia removal in MBR especially in some stressful conditions. The results showed that membrane fouling was effectively alleviated by QQ in all conditions. For the short HRT (3.94 h), the ammonia removal in QQ-MBR was fluctuating. In the presence of nitrification inhibitors (acetonitrile and allylthiourea) or at low temperature (10 °C), QQ induced much more significant suppression on nitrification in batch test and MBR. The number of the ammonia oxidizing bacteria (AOB) was not decreasing in these situations, which indicated that QQ only suppressed the activity of AOB. In all, comprehensive considerations should be taken into account when applying a QS tuning strategy to a bioreactor.

1. Introduction

Membrane bioreactor (MBR) technology has experienced a fast development for nearly three decades due to its high efficiency, small footprint and less sludge production (Judd, 2010). Moreover, longer solids retention time (SRT) adopted in MBR enables a better nitrification, which is another merit of MBR (Judd, 2008). However, membrane fouling keeps hindering the wider application of MBR since its emergence (Le-Clech et al., 2006; Meng et al., 2009). Recently, the finding of relationship between quorum sensing (QS) and membrane fouling in MBR and subsequent emergence of

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various quorum quenching (QQ) methods provided a potent and promising strategy for fouling control in MBR (Yeon et al., 2009a; Oh et al., 2012; Jiang et al., 2013; Kim et al., 2015; Lee et al., 2016b; Weerasekara et al., 2016).

QQ approaches developed for fouling control in MBR are mainly based on directly adding or indirectly enriching functional bacteria or enzymes that degrade signal molecules to suppress QS and biofilm formation, and thus to mitigate membrane fouling (Yeon et al., 2009b; Kim et al., 2013; Lee et al., 2016a, 2016c; Yu et al., 2016a). QS is the regulation of group behaviors in response to fluctuations in cell-population density (Miller and Bassler, 2001). QS bacteria release and sense signal molecules that increase in concentration as a function of cell density. The detection of a minimal threshold concentration of signal molecule triggers group behaviors, such as biofilm formation (Miller and Bassler, 2001; Shrout and Nerenberg, 2012). The physiological activities above are actually bacterial strategy for survival under environmental stresses (Dickschat, 2010). Biofilm formation allows a greater access to nutrients, an increase in resistance toward biocide, and a resistance to protozoan grazing (Davies et al., 1998; Costerton and Stewart, 2001; Matz et al., 2004). Thus, QS is of great importance for bacteria to survive and function especially under stressful conditions. All these considerations encourage us to wonder whether the QQ approaches adopted in MBR to control membrane fouling will simultaneously attenuate other QS bacteria that may be crucial for the performance of the bioreactor.

Nutrient removal has always been the priority task of a bioreactor for wastewater treatment. Nitrogen removal in bioreactor is achieved by nitrification, denitrification or anaerobic ammonium oxidation (ANAMMOX) process. Surprisingly, functional bacteria involved in the processes above were reported to be related to QS (Batchelor et al., 1997; Burton et al., 2005; Toyofuku et al., 2007; Mellbye et al., 2015; Tang et al., 2015; Huang et al., 2018). The oxidation of ammonia to nitrite, which is performed by ammonia oxidizing bacteria (AOB), is the first step and usually the rate limiting step of nitrification. QS signal molecule (acyl homoserine lactone, AHL) synthase and its gene were found in AOB (Gao et al., 2014). AOB was found to excrete AHL (C6-homoserine lactone (HSL), C8-HSL) (Burton et al., 2005). Besides, exogenous addition of AHL improved AOB activity (Batchelor et al., 1997). These indicated a strong relationship between AOB and QS (Geets et al., 2006). Thus, it is reasonable to suspect that QQ, which can suppress QS, may compromise AOB activity and ammonia removal (Geets et al., 2006).

Nitrification is a rather susceptible process in wastewater treatment. Various stressful conditions in real wastewater treatment plant can impair nitrification, like short hydraulic retention time (HRT), the presence of nitrification inhibitor and low temperature. Short HRT could occur when influent flow suddenly rise and exceed the design flow in WWTP. Some nitrification inhibitors including acetonitrile (ACN), which was reported to be contained in industrial wastewater at a high concentration (300−1400 mg/L) (Li et al., 2008), and allylthiourea (ATU, a standard inhibitor of ammonia oxidation adopted in many studies) (Drews et al., 2007) are frequently found in the inflow of real municipal WWTPs (König et al., 1998, 1999; Pagga et al., 2006). Moreover, low temperature, which is commonly encountered in winter, is another well-known unfavorable condition for AOB. Since QS is a bacteria physiological strategy for survival under environmental stresses (Dickschat, 2010), it is suspected that QQ may compromise ammonia removal more seriously under the stressful conditions above. However, to our best knowledge, systematic investigations regarding the effects of QQ on ammonia removal in MBR under common situation and stressful conditions aforementioned are still lacking.

Therefore, the aim of this study was to investigate the effect of QQ on ammonia removal in MBR, and some stressful conditions, i.e. short HRT, the presence of nitrification inhibitors (ACN and ATU), and low temperature (10 °C) were examined. Batch tests were conducted to examine the ammonia removal in activated sludge with exogenous AHL, QQ enzyme or QQ bacteria. The continuous lab scale MBR tests were conducted to investigate the effect of QQ on ammonia removal.

2. Material and methods

2.1. Chemical reagent

N-hexanoyl-DL-homoserine lactone (C6-HSL) and *N*-octanoyl-DL-homoserine lactone (C8-HSL) were purchased from Sigma-Aldrich. The stock solutions were prepared by dissolving AHLs into ACN at a concentration of 1 g/L (with 0.1% v/v formic acid) and stored at −20 °C. Acylase I from porcine kidney was purchased from Sigma-Aldrich, and 1 g/L stock solution was made with Milli-Q water and store at 4 °C. Gamma-caprolactone (GCL), ACN, ATU were purchased from Sigma-Aldrich.

2.2. Batch test of ammonia removal by activated sludge

Ammonia removal tests were conducted by mixing 15 mL domestic sewage and 15 mL activated sludge in 50 mL conical flasks, and fixing the conical flasks into the thermostatic incubator with shaking at 150 rpm. The shaking enabled the oxygen to transfer from air into the activated sludge. The dissolved oxygen (DO) in the activated sludge in the batch test was always higher than 4.5 mg/L, which was comparable to the DO in the MBRs. Domestic sewage was collected from a sewage pipe in the campus of Harbin Institute of Technology and filtered through a filter paper (Ø12.5 mm, pore size ~11 μm, Jiaojie Co. Ltd, China) prior to the experiment. The ammonia concentration in the domestic wastewater was approximately 56.8 ± 5.4 mg/L, and the chemical oxygen demand (COD) was 278.7 ± 53.4 mg/L. Activated sludge used was collected from a lab scale MBR that was fed with the same sewage, and its MLSS was around 6000 mg/L. The concentration of ammonia nitrogen in the activated sludge suspension liquid was negligible, whereas the initial concentration in the ammonia removal tests was 28.1 ± 2.3 mg/L. Since C6-HSL and C8-HSL could be produced by AOB (*Nitrosomonas europaea*) and were also commonly found in MBR, they were chosen to examine the effect of QS on nitrification (Burton et al., 2005; Yeon et al., 2009a; Yu et al., 2016a). 1 mg/L C6-HSL and 1 mg/L C8-HSL were spiked into the flask. The solvent of C6-HSL and C8-HSL was ACN, which could inhibit nitrification (König et al., 1998). In order to exclude the interference of ACN, 30 μL C6-HSL and 30 μL C8-HSL stock solution were first added into a 50 mL conical flask. The solvent was then totally evaporated using nitrogen. After that, the pre-filtered sewage, activated sludge and other ingredients were added successively into the flask.

In order to investigate QQ on nitrification, a commonly used QQ enzyme (i.e. acylase I from porcine kidney) and QQ consortia were added into the batch test. 300 μL acylase stock solution was added with a final concentration of 10 mg/L (Yeon et al., 2009a). QQ consortia was prepared by enriching activated sludge with GCL according to our previous study (Yu et al., 2016a). The AHL degradation activity of QQ consortia can be found in Yu et al. (2016a). The QQ consortia were washed with 0.8% salt solution, and then the pellets were added into the activated sludge.

Effect of QQ on nitrification was also examined in the presence of nitrification inhibitors and under low temperature condition. ACN and ATU were used as the nitrification inhibitors in this study.