



Effect of nitrifiers community on fouling mitigation and nitrification efficiency in a membrane bioreactor

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ARTICLE INFO

Keywords:

Autotrophic bacteria
Biological treatment
EPS
Biofouling
Nitrification
SMP
Wastewater

ABSTRACT

Membrane fouling in membrane bioreactors (MBR) normally depends on the microbial cell density and microbial population structure. A nitrifying-enriched activated sludge (NAS) was obtained in this study through particular ammonium feeding of conventional activated sludge (CAS). Next, the dominance of autotrophic nitrifier population in NAS system was checked and compared with CAS system by its high nitrification efficiency (100% vs. 43%) and low COD removal (9% vs. 65%). Furthermore, the maximum amount of N-NO_3^- produced from similar concentrations of ammonium in CAS and NAS systems were 6.6 mg/L and 37.5 mg/L, respectively. A filterability test also was done in the cross-flow and simple dead-end filtration systems proportionally employing different amount of NAS and CAS with a constant MLSS concentration of 2000 mg/L. NAS was twice as filterable compared to CAS. Soluble microbial products (SMP) and extracellular polymeric substance (EPS) in CAS were significantly higher than NAS system (2 and 6 mg/L for NAS vs. 100 and 36 mg/L for CAS). By increasing the proportion of nitrifying bacteria, the permeation was enhanced remarkably about 2.5 folds and the operation time of MBR was approximately doubled (6–11.5 h). The results indicated that an appropriate C/N ratio can control the microbial population and help the nitrifier significantly mitigate fouling in MBR.

1. Introduction

Due to their quality, compact size, removal efficiency, and the least amount of daily waste activated sludge, membrane bioreactor (MBR) seems to be an alternative way to apply water reuse applications in wastewater treatment [1–4]. Fouling in the membrane is regarded as a major obstacle which significantly increases the maintenance and operation costs [5–7]. Also, deposition of the cell on the membrane, excretion of extracellular polymeric substance (EPS), and soluble microbial products (SMP) cause severe biofouling [8].

Filterability can greatly be influenced by the metabolites produced by sludge on the membrane. The variables deemed to determine filterability are categorized as the constituent of wastewater [9], organic loading rate [10], solid retention time (SRT) [11,12], dissolved oxygen (DO) [13], presence of filter aids [14], flux enhancing chemicals [15], and temperature [16]. Other activated sludge parameters, such as mixed liquor suspended solids (MLSS), SMP, EPS, relative hydrophobicity, dynamic viscosity [6], and floc size [17] correlate with fouling. However, the influence of microbial community structure has less taken into account in the literature. As autotrophic microorganisms

act differently than heterotrophic ones to produce metabolites in the activated sludge, investigation on their community could introduce new approach to improve filterability in MBR. Nitrification is an aerobic process performed by nitrifying bacteria that in comparison with heterotrophs release different metabolites in the activated sludge. Heterotrophs as a rival for autotrophs, gain the space, nutrients, and oxygen in the high organic loading rates leading to a reduction in nitrifier and a change in microbial population [17,18]. Large amounts of waste activated sludges are produced during aerobic heterotrophic metabolism [19]. Accumulation of heterotrophic bacteria and their wastes prevents the activity of *Nitrosomonas* and *Nitrobacter* [20]. Van Hulle et al. [21] reported that transport of ammonia from water phase to the nitrifier cells hampered by the presence of crowded cells of heterotrophic bacteria. Heterotrophs assimilate ammonia and consume oxygen before reaching nitrifiers. In the presence of high amount of organic loading rates, nitrifiers outcompeted by heterotrophic bacteria. Therefore, the variables like the composition of raw wastewater within C/N ratio (organic carbon to nitrogen loading rates) usually affect their specific proportion [22–24]. In addition, within the low C/N ratio, the nitrifying bacteria population is proportionally up to 10 times more

Abbreviations: AOB, ammonium oxidizing bacteria; CAS, conventional activated sludge; EPS, extracellular polymeric substance; NAS, nitrifying enriched activated sludge; NOB, nitrite oxidizing bacteria; SMP, soluble microbial products; VCAS, validation of conventional activated sludge; VNAS, validation of nitrifying enriched activated sludge

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<https://doi.org/10.1016/j.cep.2018.04.006>

Received 28 December 2017; Received in revised form 29 March 2018; Accepted 2 April 2018

Available online 05 April 2018

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than the high C/N ratio [25].

The present study attempts to provide with more clarification on the role of nitrifying bacteria present in activated sludge of a membrane bioreactor and scrutinize the ways to reduce biofouling through nitrifying bacteria enrichment. Thus, the nitrifying population was enriched in the activated sludge to examine fouling and treatment efficiency based on zero to 100% proportions of nitrifiers.

2. Materials and methods

2.1. Culturing system and enrichment of nitrifying bacteria in activated sludge

The aerobic activated sludge was taken from a local municipal wastewater treatment plant. Two bioreactors with an operational volume of 20 L were utilized for the cultivation of activated sludge. CAS was used as the bioreactor containing conventional activated sludge, and NAS which was utilized to develop nitrifier-enriched activated sludge. Both bioreactors were inoculated by a similar activated sludge population. The bioreactors were opaquely selected in color and material to chemically measure the energy supply in the activated sludge. Moreover, air blower aeration was provided to be connected to an air distributor for uniform aeration in both systems. Each system was also equipped with a heater rod to keep the temperature around 30 °C, as suggested by Gerardi [26]. Also, an influent C/N ratio of zero was applied by a feed containing only mineral chemical compounds to accelerate the growth of nitrifying bacteria in NAS.

According to Table 1, glucose was added, as the source of COD to CAS. However, the C/N ratio should be zero in NAS to significantly enrich the nitrifier. The experiments were conducted for 75 days to support the sufficient growth of nitrifying bacteria. As shown in Table 1, on the first day of every week, nitrogen (N-NH_4^+) and phosphorus (P-PO_4^{3-}) were measured and maintained for the CAS and NAS and COD was measured and maintained for the CAS to provide the initial amount of synthetic wastewater.

The present medium compositions were selected regarding the average amount of N-NH_4^+ in the municipal wastewater. It should be mentioned that the pH values of these systems were measured every day to guarantee sufficient alkalinity for microorganisms; otherwise, NaHCO_3 was added to the systems. Meanwhile, the samples were taken every day at the same time to assess the variables in both systems. The initial part of the experiments focuses on developing microbial population in NAS supporting nitrifying bacteria. The CAS was the reference system during these 75 days. The experiments in this phase were conducted in batch conditions to significantly discriminate NAS from the CAS in terms of the nitrifier population.

2.2. Experiments for validation of the supposed NAS

The second part of the experiment sought to validate enrichment of nitrifier in the NAS. To do this, a specific volume of activated sludge from NAS and CAS were transferred to the other vessels with an operational volume of 5 L i.e., VNAS (Validation of nitrifier-activated sludge), and VCAS (Validation of conventional activated sludge), respectively. Feed of VNAS was similar to CAS to examine the nutrient removal and filterability of the activated sludge. Similar concentration

Table 1
Composition of synthetic wastewater used for feeding NAS and CAS cultures.

Compound	NAS feed concentration (mg/L)	CAS feed concentration (mg/L)
N-NH_4^+ as $[(\text{NH}_4)\text{SO}_4]$	40	40
P-PO_4^{3-} as $[\text{NaH}_2\text{PO}_4\cdot\text{H}_2\text{O}]$	8	8
COD	–	550

and materials (Nitrogen, Phosphorus and COD) of the feed were used in CAS and VCAS. Conduction of this phase of experiments took 14 days.

2.3. Evaluation of the fouling in the presence of the nitrifier

A series of experiments were conducted to evaluate the influence of autotrophic nitrifying bacteria on biofouling. Thus, different proportions of activated sludge taken from the NAS were combined with the CAS and filterability efficiency was evaluated by a cross-flow filtration system. Then, the following well-known equation was used to calculate the permeated flows in the simple dead-end system, which contained a paper filter (Double rings, 102, 9 cm):

$$F = \frac{V}{A \cdot t}$$

Meanwhile, a simple dead-end system was designed to measure the activated sludge filterability in different NAS proportions so that the influence of flow direction on biofouling became known. Sludge samples were also transferred to an 8 L submerged MBR as a series of filterability tests. The cross-flow system had a floating module made of a cellulose acetate membrane with a pore size of 0.4 μm and working area of 23.75 cm^2 . Moreover, the cross-flow MBR, as a filtration apparatus, was used to evaluate the variations in permeate flux being applied to the industry. Also, the operation pressure varied as much as 0.4 bar during the experiments while the temperature was held constant through the use of a heater rod. To ensure the comparability of the results in the fouling test, an identical MLSS concentration (2000 mg/L) was examined for each sludge mixture samples in the fouling tests. The cross-flow process continued to complete fouling (permeate flux which is undetectable), and the permeate flux was monitored every thirty minutes.

2.4. Measurement of different parameters

The concentrations of COD, MLSS, N-NH_4^+ , and N-NO_3^- were measured based upon the standard methods to examine water and wastewater [27]. SMP and EPS are characterized by two protein parts (EPSp, SMPp) and two carbohydrate parts (EPSc, SMPc). All four products were measured throughout the operation of the bioreactor according to the procedure presented by Le-Clech et al. [28]. After centrifuging MLSS samples at 5000g for 5 min and filtration of the supernatant, the amount of SMP in both forms was also calculated. Deionized water was then added to the sludge sample, and the mixing process was done for 10 min. Subsequently, the processes of heating and centrifuging were conducted for 10 min at 80 °C and at 7000g for 10 min, respectively. The amount of EPS in both forms was finally calculated by supernatant filtration.

3. Results and discussion

3.1. Microbial population evolution

A COD-free feed for NAS was utilized to effectively enrich the nitrifier. The single most striking observation to emerge from the data comparison was the ammonium removal in NAS and CAS. As Fig. 1 exhibits, N-NH_4^+ concentration was readjusted around 40 mg/L in the beginning of each week. Ammonium nitrogen concentration in the NAS decreased to around 25 mg/L until the third week; the ammonium consumption has, then, significantly been accelerated since the fourth week. As a consequence, the total amount of N-NH_4^+ was removed in the last four weeks while CAS reached to the maximum amount of ammonium removal of 43%. It indicates that since the fourth week, the microbial population of sludge in NAS and CAS systems became significantly different, particularly within their potential for ammonium removal. Besides, Fig. 1a shows a drop after the 28th day continuing until the 56th day while no such sharp drop was observed in the CAS

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