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A novel A-B process for enhanced biological nutrient removal in municipal wastewater reclamation

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HIGHLIGHTS

- A strategy towards sustainable nitritation was developed and demonstrated.
- Simultaneous N and P removal was achieved at low energy consumption.
- More than 88% of the total nitrogen was removed via nitritation and denitritation.
- *Accumulibacter* were the dominant PAOs with undetectable *Competibacter*.
- The A-B process offered a cost-effective option for biological nutrients removal.

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ABSTRACT

This study developed an innovative A-B process for enhanced nutrients removal in municipal wastewater reclamation, in which a micro-aerated moving bed biofilm reactor served as A-stage and a step-feed sequencing batch reactor (SBR) as B-stage. In the A-stage, 55% of COD and 15% of ammonia nitrogen was removed, while more than 88% of the total nitrogen was removed via nitritation and denitritation, together with 93% of phosphorous removal at the B-stage where ammonia oxidizing bacteria activity was significantly higher than nitrite oxidizing bacteria activity. Meanwhile substantial phenotype of polyphosphate accumulating organisms (PAOs) was also observed in the B-stage SBR. Fluorescence *in situ* hybridization revealed that *Accumulibacter* was the dominant PAOs with undetectable *Competibacter*. Compared to the conventional activated sludge process, the proposed A-B process could offer a more cost-effective alternative for enhanced biological nutrients removal from municipal wastewater with less energy consumption.

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1. Introduction

The biological nutrients removal (BNR) from municipal wastewater has been a challenge due to high energy consumption and requirement of external carbon source. To tackle this challenge, various technologies have been explored, among which the A-B processes where A-stage is designed to capture COD from municipal wastewater, and B-stage is for the nutrients removal, appear to be more promising and feasible (Wan et al., 2016). Compared to full nitrification-denitrification, nitritation-denitritation process has obvious advantages of savings in both energy- and external carbon-associated costs (Xu et al., 2015). Sidestream nitritation-denitritation processes have been implemented for treating high-strength digester liquor (Lackner et al., 2014). However, mainstream nitritation-denitritation is still under development due to the challenge in maintaining a sustainable microbial community which favors retention of ammonia oxidizing bacteria (AOB) against nitrite oxidizing bacteria (NOB). Some operation strategies promoting sustainable mainstream nitritation have been reported, such as intermittent aeration (Blackburne et al., 2008), low dissolved oxygen (e.g. DO < 2 mg L^{-1}) (Regmi et al., 2014; Zeng et al., 2014) and short sludge retention time (e.g. SRT <10 days) (Regmi et al., 2014). Evidence suggests that intermittent aeration that creates alternating anaerobic and aerobic conditions may suppress

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NOB activity when coupled with low DO and short SRT (Gilbert et al., 2014). However, it is still highly challenging for sustaining a stable mainstream nitritation-denitritation due to the lack of integrated strategy for effectively suppressing NOB.

Enhanced biological phosphorus removal (EBPR) has been considered as a sustainable process for phosphorus removal from municipal wastewater. Alternating anaerobic/oxic (A/O) conditions in temporal or spatial fashion are essentially required for promoting the growth of polyphosphate accumulating organisms (PAOs). During EBPR, volatile fatty acids (VFAs) are first converted to as poly-β-hydroxyalkanoates (PHA) in anaerobic phase which is subsequently utilized as the energy source for phosphorus up-take in oxic phase (Carvalho et al., 2007; Flowers et al., 2009). However, this may lead to insufficient carbon source for phosphorus removal due to the competition for organic carbon between PAOs and heterotrophic denitrifiers (Guerrero et al., 2011).

In BNR process, N_2O emission is of a serious concern, which is known as a powerful greenhouse gas (Rodriguez-Caballero et al., 2015). Therefore, there is an urgent need towards a better understanding of N_2O emission from biological processes for wastewater treatment as well as a control strategy for reducing its production.

Given the situation described above, it is obvious that there is an urgent need for developing a sustainable BNR process. Therefore, this study aimed to explore a novel A-B process which integrated nitritation-denitritation with EBPR for municipal wastewater reclamation. In this process, a micro-aerated moving bed biofilm reactor (MBBR) was used as A-stage for conversion of organics to VFAs which are preferable for subsequent nitritation-denitritation and EBPR in B-stage which was a step-feed sequencing batch reactor (SBR). It is expected that this study can offer new insights into possible configurations of sustainable wastewater treatment process with less energy consumption.

2. Materials and methods

2.1. A-B process

The A-B process with a 4-L micro-aerobic MBBR as A-stage and a 4-L SBR as B-stage was developed in this study (Fig. 1). The MBBR was packed with polyvinyl alcohol (PVA)-gel beads (Kuraray AQUA, Tokyo, Japan) at a volumetric ratio of 30%. The A-stage MBBR and B-stage SBR were inoculated respectively with anaerobic and aerobic sludge from a local municipal wastewater treatment plant. In this study, synthetic wastewater in simulation for the municipal wastewater in terms of COD, ammonia and phosphate concentrations was prepared with glucose and ammonium chloride as the carbon and nitrogen sources according to the experiments design in each phase of study (Table 1). Detail composition can be found elsewhere (Xu et al., 2011). The DO and effective hydraulic retention time (HRT) in the MBBR were controlled at below 0.2 mg L^{-1} and about 2.6 h, respectively. As shown in Table 1, the experiments were designed into four phases for investigating the MBBR responses to various levels of COD, nitrogen and phosphorus.

The SBR as B-stage for BNR was operated with four step-feed/anoxic/aerobic subcycles at the respective overall HRT and SRT of about 5.5 h and 4.0–5.0 d. Each subcycle consisted of 2-min feeding, 20-min anoxic phase and 12 or 15-min aerobic phase, while the times for sludge discharge, settling and wastewater discharge were present to be 2 min, 15 min and 6 min, respectively. DO in the aerobic phases was controlled at 1.4–1.6 mg L^{-1} by an on/off solenoid valve, while the temperature was kept at 30 ± 1 °C. The B-stage SBR was operated in a step-feeding mode with four subcycles, namely SC_I, SC_{II}, SC_{III} and SC_{IV}.

2.2. Analytical methods

Volatile suspended solids (VSS), ammonia nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^-–N), nitrate nitrogen (NO_3^-–N), phosphorus and COD were measured according to standard methods (Clesceri et al., 1998). The VFA was determined by a gas chromatography (GC7890A, Angilent, Wilmington, USA) equipped with flame ionization detector. Total nitrogen (TN) was calculated as the sum of NH_4^+–N, NO_2^-–N and NO_3^-–N. To determine *in situ* activities of AOB and NOB in the SBR, mixed liquor samples were taken and filtered through a 0.45-μm filter at interval of 1 min for analysisss of NH_4^+–N, NO_2^-–N and NO_3^-–N. The AOB activity was calculated as NOx^-–N production rate, while NOB activity was calculated as NO_3^-–N production rate. In order to diminish the influence of sampling on the activated sludge system, this experiment was carried out in three separate days, i.e. day 96 for SC_I, day 97 for SC_{II} and SC_{III}, and day 98 for SC_{IV}.

The cyclic concentration profiles of nitrogen compounds, PO_4^{3-}–P and N_2O in the SBR were also determined on day 152. Volume fraction of N_2O in the total gas, shown as in ppm was monitored online using Nitrous Oxide Analyzer (Thermo Scientific™, Model 46i, Waltham, USA), which can directly indicate the N_2O emission potential. Off-gas was also separately collected and then analyzed by Nitrous Oxide Analyzer. The total gas volume of each phase was measured using gas meter. The N_2O amount was calculated by equation (1) according to gas law equation, based on the off-line monitoring data.

$$m = M \times A/22.4 \times [273/(273 + T)] \times (P/101325) \quad (1)$$

In equation (1), m is the mass of N_2O (g), P is the measured absolute gas pressure in the headspace (Pa), A is measured volume of N_2O in the headspace (m^3), M is molecular weight of N_2O, and T is the absolute temperature (°C).

The dynamic abundance of AOB, NOB and denitrifiers in the SBR were determined by qPCR. The primers sets CTO189F/CTO654R, FGPS872/FGPS1269, NSR113f/NSR1264r and nosZf/nosZ1622R were used to target 16S rRNA genes of AOB β-Proteobacteria, *Nitrobacter*, *Nitrospira* and denitrifiers (Degrange and Bardin, 1995; Kowalchuk et al., 1997; Kloos et al., 2001; Dionisi et al., 2002). Standards for qPCR were prepared by cloning the amplicons into pGEM-T-Easy plasmid (Promega, Madison, WI, USA). Linearized plasmid concentration was measured by Picogreen assay to construct a standard curve in the range of $10^2 - 10^7$ copies per reaction. The qPCR was performed on Roche LightCyler® 480 with the following conditions: denaturation at 95 °C for 5 min, 40 cycles of denaturation at 95 °C for 10 s, annealing at 55 °C for 30 s and elongation at 72 °C for 30 s. The reaction was followed by a dissociation curve acquisition by measuring fluorescence throughout a linear increase in temperature from 65 °C to 98 °C at 0.1 $°C \cdot s^{-1}$. Each 10 μl reaction consists of 10 ng template, 1X LightCycler Sybr Green Master Mix and 0.5 μM of each primer.

Fluorescence *in situ* hybridization (FISH) was performed on the microbial community with probes specific for PAOs and glycogen accumulating organisms (GAOs). Sample fixation and hybridization was carried out according to standard protocol (Manz et al., 1992). The 16S rRNA-targeted oligonucleotide probes, mixed EUB338, EUB338-II, EUB338-III for universal bacteria, mixed PAO462, PAO651, PAO846, Actino658-I and Actino658-II for PAO members in *Accumulibacter* fluorescently labeled with Cy-3 and mixed GAOQ431 and GAOQ989 for GAO members in *Competibacter* fluorescently labeled with Cy-5 were used for *in situ* detection of bacteria (IDTDNA, Singapore) (Crocetti et al., 2000, 2002; Kong et al., 2005). Microbial populations were then visualized and quantified by confocal laser scanning microscopy (LSM 880, ZEISS, Jena, Germany).

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