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Achieving efficient nitrogen removal and nutrient recovery from wastewater in a combining simultaneous partial nitrification, anammox and denitrification (SNAD) process with a photobioreactor (PBR) for biomass production and generated dissolved oxygen (DO) recycling

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ABSTRACT

This study presents a new way to achieve energy neutral wastewater treatment based on a combined nitrification, anammox, and denitrification (SNAD) process and photobioreactor (PBR) configuration with external recycling instead of aeration, and without an additional carbon source, using fixed-film-activated sludge technology (IFAS). The SNAD-PBR process achieved total nitrogen (TN) and phosphorus removal efficiencies of 90 and 100%, respectively. In addition, dissolved oxygen (DO) was controlled in the range 0.4–1.2 mg/L by the introduction of an external recycling system. The presence of microalgae to serve as a carbon source in the SNAD reactor enabled the denitrifiers to survive. When the reflux ratio was 1:3, the lower COD/N protected the activity of the anammox bacteria, not suppressed by the heterotrophic denitrifiers. Microbial community analysis by Illumina MiSeq sequencing revealed that the new environment was more suitable for *Candidatus Brocadia* when a reflux system was introduced.

1. Introduction

In 2008, a development goal for wastewater treatment plants (WWTPs) was proposed during the Global Water Research Conference (GWRC); WWTPs would be transformed into energy factories, water factories, and nutrient factories (Wang et al., 2018). Thus, research on energy efficient sewage treatment technology has become an urgent issue in recent years. The main idea of energy neutrality in wastewater treatment is to recycle organic carbon, convert it into methane, and then utilize low energy consumption technology to remove nitrogen (Khiewwijit et al., 2015). In WWTPs based on an anaerobic activated sludge process, the separate treatment of dewatering liquor, a complementary technique for upgrading, is the most commonly used process (Peng et al., 2012). Sludge dewatering liquors that contain high concentrations of nutrients like ammonia and phosphorus can cause serious eutrophication problems in receiving waters (Zhang et al., 2013). A variety of strategies have been developed for effectively removing high nitrogen concentrations (Carrera et al., 2003). Compared with traditional technologies, the simultaneous partial nitrification, anammox, and denitrification (SNAD) process can remove ammonium and reduce chemical oxygen demand (COD) of wastewater (Chen et al., 2009). Moreover, a SNAD process had successfully treated sludge digester liquor in a full-scale moving bed biofilm reactor (MBBR), and that this reactor treated up to 530 kg/d of nitrogen with removal efficiency of 70% (Xu et al., 2018). However, this requires an aeration environment because the SNAD process requires sufficient oxygen in the system that ammonia oxidizing bacteria (AOB) can oxidize ammonia to nitrite (Zheng et al., 2016b). In addition, a denitrifying phosphorus removal (DPR) process can be adopted to reduce nitrate by denitrifying phosphorus accumulating organisms (DPAO), but the phosphorus removal efficiency is low (Wen et al., 2016). Thus, a new approach combining a photobioreactor and the SNAD process (SNAD-PBR) is proposed. The application of photobioreactors in wastewater treatment has been studied extensively and they seem to provide an excellent option for removing nitrogen and phosphorus via mixotrophic assimilation (Yadav et al., 2015; Kumar and Das, 2012). In addition, microalgae are fast-growing microorganisms, and their doubling time is estimated to be less than one day (Chemodanov et al., 2017). Another promising technology is the harvesting of microalgae biomass to produce energy in the form of biogas. However, despite these advantages,

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photobioreactors still have severe technical limitations, including the poor sedimentation ability of most microalgae species and long hydraulic retention times (HRT) (3–10 d) for high nitrogen concentration that often limit the nitrification of ammonium (Pires et al., 2017).

In this study, the operation of a SNAD process combined with a photobioreactor configuration with biomass recycling was optimized to improve nitrogen and phosphorus removal without an aeration system and adding a carbon source in the SNAD process. Specifically, ammonium, nitrite, and nitrate recycled from the photobioreactor were removed in the SNAD process, and residual ammonia was removed in the photobioreactor following the SNAD reactor. The process by which the AOB and nitrite-oxidizing bacteria (NOB) consume DO provided to the SNAD reactor by the effluent refluxed from the photobioreactor is not only conducive to the metabolism of anammox bacteria (AMX), but also provides a favorable environment for anaerobic denitrifying bacteria (DNB). In addition, some of the phosphate released during sludge digestion can also be removed in the photobioreactor. Thus, combining the SNAD process with a photobioreactor system is a promising alternative to conventional treatments for nitrogen and phosphorus removal. Due to the high production efficiency of algae biomass, excess microalgae in the photobioreactor can be economical and useful as a substrate for biogas production via anaerobic digestion (Zou et al. 2018). In addition, the existence of carbon sources refluxed from the photobioreactor can prevent excess nitrite accumulation by heterotrophic-type denitrifying bacteria.

The aim of this study was to assess the nutrient removal capabilities of a SNAD-PBR system at laboratory-scale by controlling the strategies employed, using microbiological characterization, and observing reactor performance. The influence of HRT, reflux ratio, and ammonia nitrogen concentration in the influent were assessed. The nitrogen removal pathways in the suspended sludge and biofilm were investigated through measuring the activity of the biofilm and suspended sludge in batch tests. In addition, the spatial distribution and biological communities of bacteria in the SNAD process were investigated using Illumina MiSeq sequencing analysis to understand the biological basis of the SNAD process as an alternative to conventional treatment.

2. Materials and methods

2.1. Microorganisms and culture conditions

Anammox biomass collected from a cylindrical, acrylic plastic, upflow anammox reactor was used as the seed sludge in our laboratory (Wang et al., 2017). AOB obtained in aerobic activated sludge collected from the Dalian Dongtai Xiajiahe WWTP (Dalian, China) were cultivated and enriched. The green microalgae *Chlorella vulgaris* was obtained from the culture collection of the Wuhan Botanical Garden, Chinese Academy of Sciences. The microalgae were then cultured in a 5 L photobioreactor composed of transparent polyvinyl chloride pipe under indoor conditions at 25 °C until late-log phase, as described in Pan et al. (2011). Portions of the *C. vulgaris* culture were stored and preserved for future experiments once a pure culture of the algae species was obtained. Synthetic wastewater was used as influent in this study, as described in Wang et al. (2016), in a modified medium as follows (all units g/L): K_2HPO_4 (0.075), $NaWO_4{\cdot}2H_2O$ (0.05), $MgSO_4{\cdot}7H_2O$ (0.075), $CaCl{\cdot}22H_2O$ (0.025), KH_2PO_4 (0.175), EDTA (15), $CuSO_4{\cdot}5H_2O$ (0.25), H_3BO_4 (0.014), $MnCl_2{\cdot}4H_2O$ (0.99), $ZnSO_4{\cdot}7H_2O$ (0.43), $NaMoO_4{\cdot}2H_2O$ (0.22), $NiCl_2{\cdot}6H_2O$ (0.19), $NaSeO_4{\cdot}10H_2O$ (0.21), and $CoCl_2{\cdot}6H_2O$ (0.24).

2.2. Reactor system

The laboratory-scale set-up used in the experiment consisted of a reactor with integrated fixed film activated sludge (IFAS) technology connected to a photobioreactor made of polymethyl methacrylate (Fig. 1). The bioreactor had an enclosed volume of 3.5 L and a working volume of 2 L, while the photobioreactor had an enclosed volume of 10 L and a working volume of 8 L. Eight strips of LED lamps and a black canvas were arranged around the outside of the reactors to provide light at an intensity of 10,000 lx (135 $\mu Em^{-2}s^{-1}$), and to shield the bacteria from light, respectively. In the photobioreactor, the pH and temperature were controlled at 7.5 ± 0.3 and 27 °C ± 1 °C, without magnetic agitation. The pH and temperature of the bioreactor were maintained at 7.8 ± 0.2 and 39 °C ± 1 °C, while magnetic agitation was constant at 160 rad/min. In addition, 1 L/d of the microalgae was continuously added to the photobioreactor to provide sufficient substrate. The experiment lasted for 218 d, and 8 phases were performed in this study.

2.3. Reactor set-up and operation strategy

Due to the slow growth rate of the anammox microbes, non-woven carriers were applied to enhance biological retention during the anammox process. In the first phase, the total nitrogen (TN) concentration was maintained at 400 mg/L. The domestication and cultivation of anammox bacteria reached completion when the effluent NO_2^--N concentration was almost constant and little NO_3^--N was being produced in the reactor. After successful acclimation of the sludge to nitrification, 1 L of the nitrification sludge was added to the anammox reactor. The DO was kept in the range 0.5–0.7 mg/L. By changing the DO and HRT, the conditions were kept optimal until the anammox successfully adapted to the new environment. After autotrophic nitrogen removal was achieved by the interaction between the nitrite and anammox bacteria, COD was introduced into the system to start the SNAD process. Meanwhile, the DO was maintained at 0.4–0.6 mg/L. In this phase of continuous operation, the COD reduction and TN removal efficiencies were 67% and 76%, respectively. It was apparent that the SNAD filler completed the biofilm process.

The main experiment began when the SNAD reactor was using anammox bacteria, nitrate bacteria, and denitrification bacteria to remove the majority of ammonia, while the photobioreactor, with *C. vulgaris*, was used to remove the remaining ammonia and phosphorus. The experiment included eight phases (I–VIII). Operational strategies of SNAD-PBR process in different phases were shown in Table 1. During the first three phases (days 0–83), the HRT was set at 2.5 d ($HRT_{SNAD\text{-}IFAS}$ = 0.5 d, $HRT_{photobioreactor}$ = 2 d) and the nitrogen concentration increased from 400 to 800 mg/L. During the fourth and fifth phases (days 84–131), the HRT increased from 2.5 to 7.5 d ($HRT_{SNAD\text{-}IFAS}$ = 1.5 d, $HRT_{photobioreactor}$ = 6 d) and 5 d ($HRT_{SNAD\text{-}IFAS}$ = 1 d, $HRT_{photobioreactor}$ = 4 d). During the last three phases (days 132–218), the reflux ratios were controlled at 1:1, 1:2, and 1:3, respectively. The HRT was set at 5 d ($HRT_{SNAD\text{-}IFAS}$ = 1 d, $HRT_{photobioreactor}$ = 4 d) and the nitrogen concentration was maintained at 400 mg/L. Some of the *C. vulgaris* culture was centrifuged at 10,000 rad/min for 10 min and then continuously fed into the photobioreactor.

2.4. Analytical methods

Influent and effluent samples were collected daily and passed through a 0.45 μm filter before analysis. The chemical parameters COD, NH_4^+-N, NO_2^--N, NO_3^--N, and phosphorus concentration were measured using Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The DO concentration was measured using a digital portable DO meter (Multi 3430, Germany). Temperature and pH were monitored using a pH meter (WTW, Germany).

2.5. Batch tests to determine the activity of the biofilm and suspended sludge

After steady operation of the SNAD-PBR system was achieved, batch experiments were carried out to investigate the activity of the biofilm and suspended sludge. The biofilm and suspended sludge were taken from the reactor at a reflux ratio of 1:3 for both the aerobic and