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Effect of algae growth on aerobic granulation and nutrients removal from synthetic wastewater by using sequencing batch reactors

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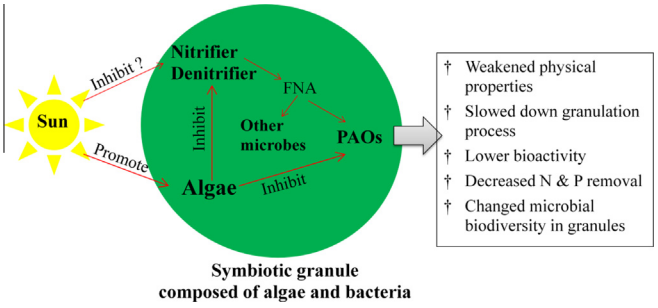
HIGHLIGHTS

- Algae growth slowed down the aerobic granulation process.
- Algal–bacterial granules had smaller particle size with loose structure.
- Granular metabolic activity was significantly decreased in the symbiosis system.
- Much less total P removal was detected in the algal–bacterial granules.
- Algal–bacterial symbiosis was unfavorable for bacteria related with N and P removal.

GRAPHICAL ABSTRACT



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ABSTRACT

The effect of algae growth on aerobic granulation and nutrients removal was studied in two identical sequencing batch reactors (SBRs). Sunlight exposure promoted the growth of algae in the SBR (Rs), forming an algal–bacterial symbiosis in aerobic granules. Compared to the control SBR (Rc), Rs had a slower granulation process with granules of loose structure and smaller particle size. Moreover, the specific oxygen uptake rate was significantly decreased for the granules from Rs with secretion of 25.7% and 22.5% less proteins and polysaccharides respectively in the extracellular polymeric substances. Although little impact was observed on chemical oxygen demand (COD) removal, algal–bacterial symbiosis deteriorated N and P removals, about 40.7–45.4% of total N and 44% of total P in Rs in contrast to 52.9–58.3% of TN and 90% of TP in Rc, respectively. In addition, the growth of algae altered the microbial community in Rs, especially unfavorable for Nitrospiraceae and Nitrosomonadaceae.

1. Introduction

Twenty years ago aerobic granular sludge was first reported by Mishima and Nakamura (1991) in a continuous upflow aerobic sludge blanket bioreactor. Up to now much effort has been put on this promising biotechnology which possesses many incomparable advantages like excellent settleability, high biomass and ability to withstand toxicity and organic loading (Adav et al., 2008a; Huang et al., 2014) in comparison to conventional activated sludge processes. So far, research works on aerobic granular sludge are mainly focused on pollutants removal efficiency and granulation mechanism in lab-scale sequencing batch reactors (SBRs), from which the results can be utilized as guidance for its practical engineering applications (Lee et al., 2010; Maszenan et al., 2011). Moreover, aerobic granular sludge with good performance for pollutants removal has been successfully cultivated in pilot-scale SBRs (Ni et al., 2009; Long et al., 2014). Therefore, in the near future, it is expected that the aerobic granular sludge technology can be applied as one of major processing units in wastewater treatment plants.

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On the other hand, natural water bodies worldwide have suffered for a long time from frequent algae blooms. Algae are also attracting researchers' attention in the field of wastewater treatment, partially because of their high capacity of nutrients uptake and oxygen production thus facilitating aeration in wastewater (Abdel-Raouf et al., 2012). Recently, the microalgal–bacterial symbiosis has been tested in ponds and photo-bioreactors, achieving cost-effective wastewater treatment (Boelee et al., 2014; Marcilhac et al., 2014). De Godos et al. (2009) used a tubular biofilm-based photobioreactor to treat pretreated swine slurry, and found that the microalgal–bacterial symbiosis could achieve nitrogen (N), phosphorus (P) and chemical oxygen demand (COD) removal efficiencies up to 100%, 90% and 75%, respectively with no external O_2 supply. Still, some researchers pointed out that the microalgal–bacterial symbiosis had some impact on the metabolism and biodiversity of microorganisms. The activity of ammonia oxidizing bacteria (AOB) was decreased by 20% with the co-existence of algae due to algae were superior competitors for N uptake compared with AOB (Risgaard-Petersen et al., 2004). In addition, Su et al. (2012) found that the bacterial communities varied with different ratios of algae to sludge inoculation in the algal–bacterial system for wastewater treatment. Up to now, however, little information can be found in the literature about the effect of algae growth on aerobic granules.

This work aimed to investigate the effect of algae growth on aerobic granulation and nutrients (COD, N and P) removal. The influences of algae on COD, N and P removal performance, and bioactivity of aerobic granules were determined. The components of extracellular polymeric substances (EPS) in addition to the changes in microbial diversity of the granules were also analyzed in order to shed light on the mechanism involved in the influence of algae growth on the aerobic granules. It is expected that this work will be useful for the cultivation and application of aerobic granules in practice.

2. Methods

2.1. Reactor set-up and operation strategy

Aerobic granules were cultivated in two identical sequencing batch reactors (SBRs) made of acrylic transparent plastic, 6 cm in diameter with a height of 60 cm. The working volume of each SBR was 1.4 L. One of the two SBRs, Rs, was placed near the window in the laboratory and irradiated around 4 h per day (from 9:00 to 13:00 due to the location of Rs) by natural sunlight from March to June, 2014. During the 100 days' operation, 61 days were sunny and the average visible light and UV-light intensity were 42 and 3 mW/cm^2, respectively during the irradiation period. Another SBR (Rc), without sunlight irradiation, was used as control.

The two reactors, namely Rs and Rc, were operated sequentially in a 4-h cycle at room temperature (25 ± 2 °C): 2 min of influent filling, 28 min of non-aeration period, 185–200 min of aeration, 5–20 min of settling, and 5 min of effluent discharge. The settling time was gradually decreased from 20 to 5 min due to the increase in settleability of the sludge. The volumetric exchange ratio was kept at 50%, leading to a hydraulic retention time of 8 h. The airflow rate was 2.0 cm/s and controlled via a gas-flow controller to keep the dissolved oxygen (DO) level between 7 and 9 mg/L in each aeration cycle.

2.2. Seed sludge and synthetic wastewater

Each reactor was inoculated with 0.5 L of seed sludge sampled from a sedimentation tank of the Shimodate Sewage Treatment Plant, Ibaraki Prefecture, Japan. On the sampling day the concentrations of chemical oxygen demand (COD), ammonia nitrogen (NH_4-N), and orthophosphate phosphorus (PO_4-P) were about 200, 30, and 3 mg/L in the plant influent, respectively. The treatment plant was under normal operation with high efficient COD, NH_4-N, and PO_4-P removals of 89–95%. The seed sludge was dark brown in color before the start-up of granulation. The initial mixed liquor suspended solids (MLSS) concentration was 3.8 g/L with sludge volume index (SVI) of 87 ml/g and MLVSS/MLSS of 0.8 in the two reactors. After aerobic granules appeared, the mixed liquor was withdrawn daily from the reactors in order to keep their solids retention time (SRT) around 20 days.

Synthetic wastewater was used in this study, and its composition was as follows: COD 600 mg/L (50% of which was contributed by glucose and sodium acetate, respectively); 10 mg PO_4-P/L (KH_2PO_4); 100 mg NH_4-N/L (NH_4Cl); 10 mg Ca^{2+}/L ($CaCl_2$); 5 mg Mg^{2+}/L ($MgSO_4{\cdot}7H_2O$); 5 mg Fe^{2+}/L ($FeSO_4{\cdot}7H_2O$); and 1 ml/L of trace element solution. The trace element solution contained (in mg/L) H_3BO_3 (50), $ZnCl_2$ (50), $CuCl_2$ (30), $MnSO_4{\cdot}H_2O$ (50), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (50), $AlCl_3$ (50), $CoCl_2{\cdot}6H_2O$ (50), and $NiCl_2$ (50) (Adav et al., 2008b). The pH in the reactors was adjusted with sodium bicarbonate to be within 7.0–8.3.

2.3. Analytical methods

Mixed liquor (volatile) suspended solids (ML(V)SS), sludge volume index (SVI), COD, NH_4-N, nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), and phosphorus (PO_4-P) were measured in accordance with the standard methods (APHA, 1998). Total concentration of phosphorus in the liquid was determined with molybdenum blue method after digestion by potassium persulfate at 120 °C. Dissolved oxygen (DO) concentration in the bulk liquor was measured with a DO meter (HQ40d, HACH, USA). pH was determined by a pH meter (Mettler Toledo FE20, Switzerland).

The microbial activity of activated sludge was indicated by specific oxygen uptake rate (SOUR), in terms of milligrams of oxygen consumed by per gram of sludge per hour. In this study, SOUR was determined at 25 °C in a 100-ml volumetric flask, which was filled with 20 ml of the mixed liquor taken from the SBR at the end of the operational cycle and 80 ml synthetic wastewater, and then sealed after insertion of a DO electrode (HQ40d, HACH, USA). The mixed liquor was agitated using a magnetic stirrer. DO level in the bulk liquor of the volumetric flask was continuously recorded by the DO meter. SOUR value was obtained by linear regression of the DO concentrations over time divided by the constant concentration of MLSS.

Batch tests were performed under DO $\geqslant$ 8.0 mg/L at 25 °C in order to obtain the maximum specific ammonium uptake rate (SAUR) and specific ammonium nitrite uptake rate (SNUR). Before testing, granules were taken from the two reactors respectively at the end of the operational cycle and aerated for 1 h to ensure that all ammonium ions were completely consumed and converted. Subsequently, the granules were washed with tap water, and then divided into three aliquots based on wet weight. Each aliquot was dosed into one 250 ml flask filled with the same synthetic wastewater used in this study (except for ammonium or nitrite concentration). A pulse of concentrated stock solution of ammonium or nitrite was added at the beginning of test in order to achieve an initial concentration of 50 or 20 mg-N/L, respectively. Samples were collected at an interval of 20 min and then measured. Granular SAUR or SNUR was obtained by linear regression of the NH_4-N or NO_2-N concentrations over time divided by the constant concentration of MLSS.

Extracellular polymeric substances (EPS) were extracted from the sludge by using ultrasound-formaldehyde-sodium hydroxide method (Adav and Lee, 2008). Extracellular proteins (PN) in the extracted EPS were determined by Bradford method with bovine