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Understanding the impact of influent nitrogen concentration on granule size and microbial community in a granule-based enhanced biological phosphorus removal system

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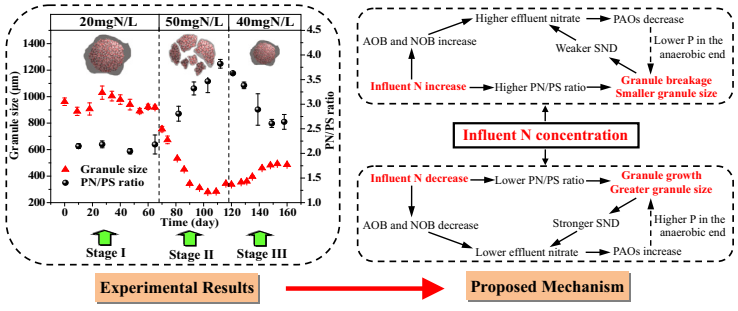
HIGHLIGHTS

- Influent N concentration affects granule size in a granule-based EBPR system.
- PN/PS ratio in EPS plays a crucial role in granule stability.
- Influent N concentration affects microbial community and activity.
- Mechanism is proposed for the effect of influent N concentration on granule size.

GRAPHICAL ABSTRACT



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ABSTRACT

To better understand the effect of influent nitrogen concentration on granule size and microbial community in a granule-based enhanced biological phosphorus removal system, three influent nitrogen concentrations were tested while carbon concentration was an unlimited factor. The results show that although ammonium and phosphate were well removed in the tested nitrogen concentration range (20–50 mg L^{-1}), granule size, the amount of phosphate accumulating organisms (PAOs) and microbial activity were affected significantly. A possible mechanism for the effect of influent nitrogen concentration on granule size is proposed based on the experimental results. The increase in proteins/polysaccharides ratio caused by high influent nitrogen concentration plays a crucial role in granule breakage. The small granule size then weakens simultaneous nitrification–denitrification, which further causes higher nitrate concentration in the effluent and lower amount of PAOs in sludge. Consequently, phosphate concentration in the anaerobic phase decreases, which plays the secondary role in granule breakage.

1. Introduction

Aerobic granular sludge (AGS) process is an attractive technology for biological nutrient removal from wastewater since it can greatly reduce the plant footprint and capital cost due to the dense and compact structure and the fast settling velocity (de Bruin et al., 2004). Extensive work have been documented on the granule characteristics, factors affecting granulation and granule stability, response of granules to various environmental and operational conditions, and granulation mechanisms (Show et al., 2012). However, instability of the matured granules is still one of the challenges for the scaling up of AGS reactors.

To date, many researches on nutrient removal AGS systems have focused on nitrogen (N) removal as nitrification and

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denitrification can be achieved simultaneously owing to the dissolved oxygen (DO) gradient within the granules. Recently, however, it is reported that AGS was easily formed in an enhanced biological phosphorus removal (EBPR) system even though the operational parameters and reactor configuration did not favor the granulation such as long settling time, low aeration intensity, and low height to diameter (H/D) ratio (Wu et al., 2012). Therefore, compared with the AGS system for nitrogen removal, the granule-based EBPR system has more advantages due to its lower requirement for the reactor structure and lower aeration cost associated with the formation and maintenance of AGS. However, both EBPR and biological N removal are expected in a real wastewater treatment plant due to the requirement for eutrophication control nowadays. The AGS system may offer a possibility to obtain simultaneous removals of chemical oxygen demand (COD), N and phosphorus (P) in a single reactor (de Kreuk et al., 2005; Coma et al., 2012; Weissbrodt et al., 2013). Nevertheless, Coupling N removal and EBPR could cause some detrimental interactions between both processes (Guerrero et al., 2013).

Granule stability and disintegration have been investigated by some researchers. Many factors, such as outgrowth of filamentous organisms, anaerobic degradation within granules, functional loss of strains and extracellular polymeric substances (EPS), account for the loss of granule stability in the long-term operation of an AGS system (Show et al., 2012). Among these factors, EPS secreted by microorganisms during their growth and lysis are considered to play an essential role in granule formation and maintenance (Liu et al., 2004a; Wang et al., 2006; Lin et al., 2013). As major components of EPS, proteins (PN) have a positive effect on the cell hydrophobicity and surface charge of granular sludge, which is important for the granulation and granule stability (Liu et al., 2014), and polysaccharides (PS) are deemed to be the backbone supporting the strength of granular sludge (Adav et al. 2008). Yang et al. (2005) and Luo et al. (2014) found that decreasing the COD/N ratio in the influent caused the decrease in EPS production, and the PN/PS ratio showed a decreasing trend in an AGS system for N removal. However, there has been limited consideration for the relationship between PN/PS ratio and granule stability in a granule-based EBPR system for simultaneous N and P removal. Furthermore, exact causes are still not clear for the deterioration of granule stability in this kind of systems.

Granule size has been recognized to be a simple and direct parameter to illustrate the granule stability. When the granule disintegration occurs, the granule size decreases over time. In contrast, the increase in granule size can be observed when the granules are reformed (Liu et al., 2014). Verawaty et al. (2013) demonstrated that a certain stable granule size (the critical size) was influenced by the process conditions, including wastewater characteristics, aeration, reactor geometry, mixing, and solid concentration. Food to microorganism ratio and the hydrodynamics during the mixing phase are the two parameters influencing the granule size, which were studied a lot in an AGS system for COD and N removal (Tay et al., 2004; Li et al., 2011). Furthermore, COD/N ratio also plays an important role in granule size in an N removal AGS system. Yang et al. (2005) reported that the mean granule size declined significantly from 1.5 mm to 0.5 mm and 0.4 mm when COD/N ratio decreased from 10 to 5 and 3.3, respectively. Recently, Luo et al. (2014) also found the similar trend when the COD/N ratio decreased from 4 to 1 in an N removal AGS system. However, it remains a question whether N concentration alone can influence the granule stability when carbon source is an unlimited factor for nutrient removal. In an AGS system for simultaneous COD, N and P removal, de Kreuk et al. (2005) observed the dependency between particle diameter and N removal efficiency, but they did not explain this phenomenon. Since N is a very important component no matter in municipal or industrial wastewaters, and removing N and P simultaneously from wastewater is an urgent task nowadays, it is of practical significance to investigate the effect of influent N concentration on granule stability in the granule-based EBPR system for simultaneous N and P removal.

The objective of this study is to gain an insight into the effect of influent N concentration on granule size and microbial community in a granule-based EBPR system for simultaneous N and P removal based on the premise that carbon source is an unlimited factor for nutrient removal. Three influent N concentrations were carried out in a granule-based EBPR system with the influent COD/N ratio higher than 8. The granule size, PN and PS contents in EPS, P content in sludge, evolution of microbial community and reactor performance were monitored during the experimental period. A possible mechanism is proposed for the effect of influent N concentration on granule size based on the experimental results. This can aid in better understanding the granulation and granule stability in a granule-based EBPR system for simultaneous N and P removal.

2. Methods

2.1. Reactor setup and operation

A lab-scale AGS sequencing batch reactor (SBR) was used. The effective H/D ratio of the reactor was 3.0; the working volume was 15 L, and the volumetric exchange ratio was set at 51%. Bottom aeration was supplied with an airflow rate of 180 L h^{-1} (corresponding superficial air velocity = 0.16 cm s^{-1}). The reactor was placed in a temperature controlled room at 20 ± 2 °C. Mixed liquor was withdrawn daily from the reactor to maintain the sludge retention time (SRT) at 20 days. The SBR was operated on a 6-h cycle consisting of static feeding (4 min), anaerobic phase (90 min), aerobic phase (180 min), anoxic phase (60 min), settling (8 min) and decanting (18 min). Synthetic wastewater with the following composition was used: 512.5 mg of sodium acetate (400 mg L^{-1} as COD basis), 43.9 mg of KH_2PO_4 (10 mg L^{-1} as PO_4^{3-}-P basis), 76.4–191.1 mg of NH_4Cl (20–50 mg L^{-1} as NH_4^+-N basis), 123 mg of $MgSO_4{\cdot}7H_2O$ (12 mg L^{-1} as Mg basis, about 10 mg L^{-1} Mg from tap water), 125 mg of $CaCl_2$ (45 mg L^{-1} as Ca basis, about 40 mg L^{-1} Ca from tap water) and 0.3 mL of trace elements solution (Kishida et al., 2006).

2.2. Experimental design

2.2.1. SBR experiments

The AGS SBR operated under anaerobic–aerobic–anoxic conditions for biological N and P removal had been stably running for more than 3 months before this study. During this period, P-accumulating microbial granules with the mean size of 1100 μm were dominant in the reactor. The influent N concentration was maintained at 15 mg L^{-1}, and the average removal efficiencies of 93.6%, 98.9%, 82.3%, and 99.4% were achieved for COD, ammonium, total nitrogen (TN), and phosphate, respectively. In the present study, three stages (I, II and III) were carried out to investigate the effect of influent N concentration on granule size and microbial community. All the operational conditions for the three stages were consistent with the setup stage except for the influent N concentration. Specifically, the influent N concentration for stage I, II and III were 20 mg L^{-1}, 50 mg L^{-1}, and 40 mg L^{-1}, respectively; the corresponding COD/N ratios were 20, 8, and 10, respectively. In order to reach the steady state of the reactor, each stage was run approximately 50 days (i.e. 2.5 SRTs). Typical cycle tests and batch experiments for microbial activity measurement were carried out at the end of each stage to evaluate the performance of the system and evolution of the microbial population. Samples for fluorescence in site hybridization (FISH) were also collected at the end of each stage.