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Identification of inorganic and organic species of phosphorus and its bio-availability in nitrifying aerobic granular sludge

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ABSTRACT

Phosphorus (P) recovery from sewage sludge is necessary for a sustainable development of the environment and thus the society due to gradual depletion of non-renewable P resources. Aerobic granular sludge is a promising biotechnology for wastewater treatment, which could achieve P-rich granules during simultaneous nitrification and denitrification processes. This study aimed to disclose the changes in inorganic and organic P species and their correlation with P mobility and bio-availability in aerobic granules. Two identical square reactors were used to cultivate aerobic granules, which were operated for 120 days with influent ammonia nitrogen (NH_4–N) of 100 mg/L before day 60 and then increased to 200 mg/L during the subsequent 60 days (chemical oxygen demand (COD) was kept constant at 600 mg/L). The aerobic granules exhibited excellent COD removal and nitrification efficiency. Results showed that inorganic P (IP) was about 61.4–67.7% of total P (TP) and non-apatite inorganic P (NAIP) occupied 61.9–70.2% of IP in the granules. The enrichment amount of NAIP and apatite P (AP) in the granules had strongly positive relationship with the contents of metal ions, i.e. Fe and Ca, respectively accumulated in the granules. X-ray diffraction (XRD) analysis and solution index calculation demonstrated that hydroxyapatite ($Ca_5(PO_4)_3(OH)$) and iron phosphate ($Fe_7(PO_4)_6$) were the major P minerals in the granules. Organic P (OP) content maintained around 7.5 mg per gram of biomass in the aerobic granules during the 120 days' operation. Monoester phosphate (21.8% of TP in extract), diester phosphate (1.8%) and phosphonate (0.1%) were identified as OP species by Phosphorus-31 nuclear magnetic resonance (^{31}P NMR). The proportion of NAIP + OP to TP was about 80% in the granules, implying high potentially mobile and bio-available P was stored in the nitrifying aerobic granules. The present results provide a new insight into the characteristics of P species in aerobic granules, which could be helpful for developing P removal and recovery techniques through biological wastewater treatment.

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1. Introduction

Phosphorous (P) is an essential and limiting element for living organisms and human beings, especially for the growth of plants. P deficiency will greatly affect and restrict crop yields worldwide. However, phosphate rock, a non-renewable resource and raw material used for P fertilizers, is reported to be depleted in 50–100 years (Cordell et al., 2009). Thus P resource protection and P recovery is prerequisite for a sustainable agriculture and society on a global scale. On the other hand, sewage sludge generated from wastewater treatment is regarded as a potential phosphorus reservoir due to its high production amount and high P content (Xu et al., 2012). Land application of sewage sludge as P fertilizer has become one of the main sludge disposal practices (Singh and Agrawal, 2008; Xie et al., 2011a).

Aerobic granular sludge is a promising biotechnology for nitrogen and phosphorus removal from wastewater. The aerobic granules possess advantages like excellent settleability, high biomass and ability to withstand high loading rate compared to conventional activated sludge processes (Qin et al., 2004; Yilmaz et al., 2008; Adav et al., 2008a). Previous studies reported that P-rich aerobic granules could be achieved during simultaneous nitrification and denitrification processes (Lin et al., 2012; Li et al., 2014), indicating that aerobic granular sludge can be more prospectively used for P fertilizer after being properly treated.

As well known that not all the forms of P exhibit similar mobility and bio-availability in the sludge, detailed information about P fraction is necessary for both activated sludge and aerobic granules, especially when land application of sludge is taken into consideration. Moreover, identification of P species in aerobic granular sludge is helpful to understand the characteristics and function of P in aerobic granules and thus the mechanisms of P removal through this new biotechnology. For this purpose, some researchers declared that sewage sludge contained higher mobilizable forms of P, i.e. non-apatite inorganic P than sediment (Medeiros et al., 2005; Xie et al., 2011a; 2011b), which is much meaningful for land application of sewage sludge. To date, as for aerobic granules, lab-scale experiments have been conducted on the mineral forms of P, demonstrating that the major inorganic P species in aerobic granules are hydroxyapatite (HAP), struvite, or Ca–Mg phosphate and whitlockite ($Ca_3(PO_4)_2$), greatly dependent on influent characteristics and operation conditions (Angela et al., 2011; Lin et al., 2012; Li et al., 2014). However, the organic P species in aerobic granules, the potential available P resource closely related with the activity of phosphate accumulating organisms (PAOs) (Uhlmann et al., 1990), is poorly understood most probably attributable to its complex nature and the limitation of analytical methods.

Recent research indicates that phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P NMR) can be used for analyzing inorganic and organic P species (orthophosphate monoesters, orthophosphate diester and phosphonates) in sediments, soil and activated sludge since it is able to distinguish multiple P compounds among complex substances (Uhlmann et al., 1990; Ahlgren et al., 2011; Li et al., 2013). Therefore it is speculated that this technology could provide more insight into the P species in aerobic granules, especially the organic P fraction.

This study aims to reveal the fractionation and distribution of P in aerobic granules and to evaluate the mobility and bio-availability of P in aerobic granules. In addition, organic and inorganic P species in the granules were also determined and characterized by ^{31}P NMR and X-ray diffraction (XRD), respectively. It is expected that this work would not only be useful for P utilization and recovery from aerobic granules but also provide insight into the characteristics of P in aerobic granules, which will help to develop applicable technologies for P removal and recovery through wastewater treatment.

2. Materials and methods

2.1. Experimental set-up and operation

Aerobic granules were cultivated in two identical sequencing batch reactors (SBRs) made of acrylic plastic with height of 60 cm and square cross section of 6 cm × 6 cm. Their effective working volume was 1.4 L. Aeration was provided by an air pump (AK-30, KOSHIN, Japan) through air bubble diffusers at the bottom of each reactor with an air flow rate of 2.0 cm/s and the dissolved oxygen (DO) maintained at 7–9 mg/L during aeration. Synthetic wastewater was used in this study, and its composition was as follows: chemical oxygen demand (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) during the first 60 days' operation and then increased to 200 mg NH_4–N/L till the end of experiment; 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 7.5–8.0.

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. The initial mixed liquor suspended solids (MLSS) concentration was 4.6 g/L with sludge volume index (SVI) of 81.4 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 40 days. The reactors 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, 186–206 min of aeration, 2–20 min of settling, and 4 min of effluent discharge. The settling time was gradually decreased from 20 min to 2 min due to the increase in settleability of the sludge. The volumetric exchange ratio was kept at 54%, resulting in a hydraulic retention time of 7.4 h.

2.2. Chemical and physical analysis

Mixed liquor (volatile) suspended solids (ML(V)SS), sludge volume index (SVI), COD, ammonia nitrogen (NH_4–N), nitrite nitrogen (NO_2–N), nitrate nitrogen (NO_3–N), and phosphorus (PO_4–P) were measured in accordance with standard methods