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Simultaneous pyridine biodegradation and nitrogen removal in an aerobic granular system

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ABSTRACT

Simultaneous pyridine biodegradation and nitrogen removal were successfully achieved in a sequencing batch reactor (SBR) based on aerobic granules. In a typical SBR cycle, nitritation occurred obviously after the majority of pyridine was removed, while denitrification occurred at early stage of the cycle when oxygen consumption was aggravated. The effect of several key operation parameters, *i.e.*, air flow rate, influent NH_4^+-N concentration, influent pH and pyridine concentration, on nitritation, pyridine degradation and total nitrogen (TN) removal, was systematically investigated. The results indicated that high air flow rate had a positive effect on both pyridine degradation and nitritation but a negative impact of overhigh air flow rate. With the increase of NH_4^+ dosage, both nitritation and TN removal could be severely inhibited. Slightly alkaline condition, *i.e.*, pH 7.0–8.0, was beneficial for both pyridine degradation and nitritation. High pyridine dosage often resulted in the delay of both pyridine degradation and nitritation. Besides, extracellular polymeric substances production was affected by air flow rate, NH_4^+ dosage, pyridine dosage and pH. In addition, high-throughput sequencing analysis demonstrated that *Bdellovibrio* and *Paracoccus* were the dominant species in the aerobic granulation system. Coexistence of pyridine degrader, nitrification related species, denitrification related species, polymeric substances producer and self-aggregation related species was also confirmed by high-throughput sequencing.

Introduction

Aerobic granular sludge is drawing increasing interest from the global researchers, due to its excellent performance in terms of contaminant removal from wastewater (Adav et al., 2008a). Compared with the conventional activated sludge flocs, aerobic granules have regular shape and compact structure, resulting in the enhanced settleability, high biomass retention, multifunctional microbial community, high tolerance to toxicity and shock loading, and relatively low production of excess sludge (Maszenan et al., 2011). Because of these unique features, the aerobic granulation technology has been devoted to the treatment of high strength wastewater containing various contaminants, such as organics, nitrogen and phosphorus (Adav et al., 2007; Wei et al., 2014). Recently, considerable efforts have been directed at the cultivation of aerobic granules for the removal of recalcitrant pollutants, such as phenol, nitrobenzene, *p*-nitrophenol, pyridine and 2-fluorophenol (Adav et al., 2007, 2008a). There has been strong evidence that aerobic granules have a promising potential for

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the treatment of wide-spectrum industrial wastewater containing high-strength recalcitrant compounds (Adav et al., 2008a).

Several industries, such as the chemical, petrochemical, coke plant and refineries, often produce complex wastewaters containing both recalcitrant compounds and ammonium (Maszenan et al., 2011). Moreover, biodegradation of the recalcitrant compounds such as nitrogen heterocyclic compounds, was often accompanied by the release of ammonium (Shen et al., 2015b). Accordingly, removal of various recalcitrant compounds and ammonia from wastewater is the two primary functions of industrial wastewater treatment system (Zhang et al., 2011). Expensive physico-chemical treatment technologies, such as adsorption and advanced oxidation, were often advised prior to predenitrification based on anaerobic and aerobic process for the treatment of those wastewaters containing both recalcitrant compounds and ammonium. Nevertheless, there is no doubt that autotrophic biological nitrogen removal (BNR), *i.e.*, nitritation plus anammox, is currently regarded as the technology with the cheapest cost and the lowest environmental foot-print. BNR *via* nitrite is considered as an economic savings technology which reduces the aeration consumption by 25% during nitrification and saves the organic matter dosage by 40% and 100% for the subsequent denitrification and anammox processes, respectively (Xu et al., 2012). The key for maintaining nitritation is to selectively inhibit or washout nitrite oxidizing bacteria (NOB) over ammonia oxidizing bacteria (AOB) by several control strategies, including pH adjusting, low dissolved oxygen (DO), high free ammonia (FA) and free nitrous acid (FNA) (Wei et al., 2014; Guo et al., 2009).

Aerobic granulation is efficient to retain slow-growing or sensitive microorganisms such as AOB in the wastewater treatment system (Liu et al., 2008). Nitritation carried out in aerobic granular system is characterized by high biomass retention in pursuit of high nitrogen loads and wide diverse microbial species, which often results in robust nitritation and possible total nitrogen (TN) removal (Wang et al., 2012). The specific layer structure of aerobic granules can contribute to create concentration gradients of potentially toxic or inhibitory compounds; thus, sensitive microorganisms such as AOB can be protected from the direct acute toxicity (Maszenan et al., 2011). In this sense, some studies have shown the possibility of aerobic granules to perform simultaneous nitritation and biodegradation of some inhibitory compounds. Jemmat et al. (2013) revealed that simultaneous nitritation and *p*-nitrophenol biodegradation was successfully achieved in a single reactor based on aerobic granules, with approximately 85% ammonium removed through nitritation, despite unstable *p*-nitrophenol degradation. It was also observed that in a continuous airlift reactor bioaugmented with *p*-nitrophenol degrading activated sludge, partial nitritation was kept stable and *o*-cresol could be totally degraded, even in the *o*-cresol shock-load events (Jemmat et al., 2014).

By now, nitritation by aerobic granules has been a major concern for the efficient nitrogen removal from wastewater. However, most works were based on the wastewater containing biodegradable carbon source and low-strength ammonium. Although a continuous airlift reactor based on aerobic granules has been recently proposed for simultaneous nitritation and biodegradation of recalcitrant compounds such as *p*-nitrophenol and *o*-cresol, the available information is still very limited, and systematic investigation is still absent (Jemmat et al., 2013, 2014). Mechanisms involved in the simultaneous recalcitrant compound removal and possible nitrogen removal through nitritation and denitrification in a single aerobic granular reactor are not fully understood. Our previous study demonstrated that pyridine removal could be remarkably enhanced in an aerobic granular system bioaugmented by *Rhizobium* sp. NJUST18. However, due to the high loading of pyridine and NH_4^+ in this bioaugmented aerobic granular system, nitrification within this bioaugmented system was completely inhibited (Liu et al., 2015). Although this bioaugmented aerobic granular system could be considered as a promising way to achieve highly efficient wastewater treatment, simultaneous pyridine degradation and nitrogen removal within this bioaugmented system have not been investigated. There was a lack of comprehensive study regarding the key factors influencing the system performance in terms of nitrogen and pyridine removal.

Extracellular polymeric substances (EPS) released by the aggregated microbes are considered to play an important role in granule formation and maintenance (Sheng et al., 2010). The functions and stability of the bioaugmented system treating recalcitrant pollutants are closely related with EPS in it, since EPS production is a response to the stress situation. EPS secreted can contribute to adhesion, promoting microbial aggregation, formation of matrix structure, and enhancing the communication between cells and the stability of granules (Kong et al., 2014). However, EPS contents and components of the aerobic granules exposed to pyridine have not been carried out previously. Information on the microbes responsible for simultaneous pyridine degradation and nitrogen removal should also be revealed in detail. Mechanisms involved in the simultaneous pyridine removal and possible nitrogen removal through nitritation and denitrification in a single aerobic granular reactor are not fully understood.

In the present study, the main aim was to obtain simultaneous pyridine biodegradation and nitrogen removal in a single SBR based on aerobic granules. The effect of several key operation parameters on pyridine degradation, nitrogen removal and EPS contents was investigated. High-throughput sequencing analysis was performed for the identification of the bacteria species involved in the aerobic granules. The possible mechanism involved in the simultaneous pyridine removal and nitrogen removal was also suggested.

1. Materials and methods

1.1. Reactor setup and influent

A column-type SBR with working volume of 2.2 L and volumetric exchange ratio of 50% was used for the cultivation of aerobic granules in this study. The SBR was operated at cycle period of 8 hr:7 min of feeding without stirring, 7.6 hr of aerobic reaction, 3 min of settling, 4 min of effluent withdrawal and 10 min of idling. Aeration and mixing were supplied through an air diffuser placed at the bottom of the reactor. The temperature was maintained at 30 °C using a water bath circulator with a heating system. All the operation processes were automatically

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