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Effect of acetate to glycerol ratio on enhanced biological phosphorus removal

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HIGHLIGHTS

- Effect of acetate to glycerol ratio on the EBPR performance was evaluated.
- The optimal ratio of acetate to glycerol for EBPR was 50/50%.
- The underlying mechanism glycerol enhanced the capability of EBPR was investigated.
- The appropriate addition of glycerol increased PHA synthesis.

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ABSTRACT

Enhanced biological phosphorus removal (EBPR) is a sustainable and promising technology for phosphorus removal from wastewater. The efficiency of this technology, however, is often discounted due to the insufficient carbon sources in influent. In this work, the effect of acetate to glycerol ratio on the EBPR performance was evaluated. The experimental results showed when the ratio of acetate to glycerol decreased from 100/0% to 50/50%, the EBPR efficiency increased from 90.2% to 96.2%. Further decrease of acetate to glycerol ratio to 0/100% decreased the efficiency of EBPR to 30.5%. Fluorescence *in situ* hybridization analysis demonstrated appropriate increase of glycerol benefited to increase the relative abundance of phosphate accumulating organisms. Further investigation revealed the proper addition of glycerol increased the amount of polyhydroxyalkanoates synthesis, and then produced sufficient energy for oxic luxury phosphorus in the subsequent oxic phase.

1. Introduction

Enhanced biological phosphorus removal (EBPR) is considered as the most economical and sustainable strategy to protect the water body from eutrophication (Zhao et al., 2016a; Wang et al., 2017a). EBPR is generally achieved by alternating anaerobic and oxic conditions to promote the enrichment of activated sludge with polyphosphate accumulating organisms (PAOs), the key microorganisms associated with phosphorus removal. In anaerobic phase, PAOs are able to take up the available volatile fatty acids (VFAs) and store them as polyhydroxyalkanoates (PHAs), with the energy and reducing power from polyphosphate cleavage and glycogen degradation, respectively (Oehmen et al., 2007). In the subsequent oxic environment, the PHAs synthesized anaerobically are consumed to produce energy for luxury uptake of phosphate,

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microbial growth, and glycogen replenishment (Mielczarek et al., 2013). Thus, EBPR could be achieved by wasting P-rich sludge at the end of the oxic phase.

Generally, the performance of EBPR is satisfactory in well-defined laboratory experiments (Chen et al., 2016; Wang et al., 2014). In real wastewater treatment plants (WWTPs), however, unexpected failure of EBPR is usually observed due to the lost of PAOs or the reduced activities of PAOs. Available carbon in the raw wastewater is an important factor affecting the efficiency of biological phosphorus removal (BPR), but the carbon source of urban sewage plant in influent is often insufficient in some areas especially in the South China (Jin et al., 2014; Wu et al., 2014), which greatly reduced the performance of BPR. To solve the problem, three strategies are proposed: i) adding industrial carbon sources (e.g., acetate, propionate, and citric acid) directly (Yuan et al., 2010; Mielcarek et al., 2015; Wang et al., 2012a), ii) utilization of sludge pre-fermentation to produce volatile fatty acids (VFAs) (Tong and Chen, 2007; Yin et al., 2016a; Zhao et al., 2015a, 2016b, 2016c; Li et al., 2016; Wang et al., 2017b), and iii) process operation mode change (e.g., two-sludge system) (Kuba et al., 1996; Marcelino et al., 2011; Wang et al., 2017a). Although the above mentioned strategies can improve the content of carbon source or maximize the utilization of existing carbon sources in wastewater, they all have their own shortcomings. Society is now increasingly aware that wastewater treatment should be shifted in a more sustainable way (Li et al., 2014; Wang et al., 2015, 2017c; Zhao et al., 2018). Adding industrial production of acetate and/or propionate directly would increase the overall operating cost and carbon footprint of WWTPs, which do not support sustainable operation. Using the VFA-riched fermentation liquid to enhance EBPR is feasible in laboratory and pilot scale (Feng et al., 2009; Li et al., 2011). However, this approach requires either to remove the high concentrations of NH_4^+-N, PO_4^{3-}-P, heavy metals, and other toxicities in the fermentation liquid (Chen et al., 2018; Xu et al., 2017; Wang et al., 2017d) or to establish a side-stream short-cut sequencing batch reactor (Katsou et al., 2015; Longo et al., 2017), which is not feasible in some backward technology-equipment and small-scale WWTPs. Process improvement (such as two-sludge system) can maximize utilization of organic carbon in raw wastewater to achieve excellent biological nutrient removal, but it increases lots of infrastructure costs and covers a relatively large area, which is not economically feasible in some small-scale WWTPs as well. After assessing the above strategies comprehensively and considering the substantial amounts of wastewater treated daily, the addition of a low-cost carbon source is presented as a fast and successful solution to provide enough chemical oxygen demand (COD) for PAOs to achieve excellent phosphorus removal. Achieving a satisfactory phosphorus removal capacity not only consider the adequate amount of carbon source but also consider the nature of additional carbon sources. Some types of carbon sources contribute little to the improvement of EBPR but increase the overall operating costs and carbon footprint of the WWTPs (Isaacs and Henze, 1995; Yuan et al., 2010). Thus, a cost-efficient and technically feasible carbon source to enhance the vitality of PAOs needs to be urgently explored.

Glycerol, the by-product of biodiesel fuel production, is inevitably generated with great amounts during biodiesel refining. It was reported that every 10 L of biodiesel fuel produced could produce 1.0 L of crude glycerol (Johnson and Taconi, 2007). Crude glycerol derived from biodiesel production contains many impurities such as methanol, salts or long chain fatty acids, thus resulting in the undesirable market price of glycerol. Glycerol would pose a potential risk to environmental ecology and human health if it is not treated and disposed properly (Escapa et al., 2009; Guerrero et al., 2012; Carrera et al., 2003). The carbon content in glycerol is high (around 40% in terms of mass fraction), so it is a valuable raw material for the production of pharmaceuticals and personal care products and can be employed to serve as carbon source for wastewater treatment (Coats et al., 2015; Guerrero et al., 2015). It was reported that glycerol can be used as sole carbon source to achieve excellent biological denitrification in modified up-flow anaerobic sludge blanket reactor (Grabińska-Ńoniewska et al., 1985). Hinojosa et al. (2008) also employed glycerol as external carbon to facilitate the removal of nitrate, and the specific denitrification rate (SDNR) of acclimated mixed liquor was 1.4 mg NOx-N/g volatile suspended solids (VSS)/hr. As for the process of EBPR, Guerrero et al. (2012, 2015) investigated the feasibility of using glycerol as the sole carbon source for the removal of phosphate, and it was found that satisfactory efficiency of EBPR was achieved when the anaerobic hydraulic retention time (HRT) was appropriate. The key point was the utilization of a longer anaerobic phase to allow complete fermentation of glycerol to VFA, which maintained EBPR activity using glycerol as sole carbon source. This good attempt by Guerrero et al. (2012, 2015) opened a new range of possibilities for EBPR from carbon source deficit wastewaters. In the real sewage treatment process, however, glycerol can not be used as the sole carbon source for BPR because the wastewater contains a certain amount of inherent carbon source (mainly as acetate) (Oehmen et al., 2005). Different species of microbial populations have different abilities to compete for the available carbon source (Carvalheira et al., 2014; Zhao et al., 2016d). To date, however, the effects of glycerol together with other VFAs (mainly acetate) serving as mixed carbon source on the performance of BPR and their underlying mechanisms are rarely investigated. Although Zhao et al. (2016a) explored the effects of acetate and glycerol on the efficiencies of BPR induced by the novel oxic/extended idle regime, the impact of the ratio of acetate to glycerol on the EBPR performance in the conventional A/O regime has not been reported yet. The modes of microbial metabolism and reactor operation induced by A/O regime are quite different from those in the oxic/extended idle phosphorus removal process (Wang et al., 2008, 2012a, 2012b). In order to better understand the feasibility of glycerol as an additional carbon source for EBPR, it is necessary to explore the impact of different ratios of acetate to glycerol on the performance of EBPR.

The aim of this work was to systematically evaluate the potential impact of glycerol addition on the performance of conventional A/O regime. Firstly, the EBPR efficiency was evaluated at different ratios of acetate to glycerol during long-term operation. Then, the details and underlying mechanisms of how the ratio of acetate to glycerol affects the BPR efficiency were explored from the aspects of relative abundance of functional microorganisms (i.e., PAOs and GAOs), the transformations of metabolic intermediates in one cycle, and the activities of key enzymes. This is the first study clarifying that appropriate ratio of acetate to glycerol could improve the efficiencies of EBPR in the conventional A/O process. These findings obtained might provide one promising method for simultaneous improvement of EBPR efficiency and utilization rate of waste materials.

2. Materials and methods

2.1. Sludge inoculum and synthetic wastewater

The sludge inoculum used in this study was obtained from the secondary sedimentation tank outlet of a WWTP located in Changsha, China. The carbon source of synthetic wastewater is acetate, because it is the most abundant VFAs present in wastewater (Wang et al., 2013). The synthetic wastewater solution also consists of (mg/L in distilled water) 65.8 KH_2PO_4, 114.6 NH_4Cl, 23.9 $MgSO_4{\cdot}7H_2O$, 50 $MgCl_2{\cdot}6H_2O$, 22 $CaCl_2{\cdot}2H_2O$, and 30 mL/L nutrient solution, the compositions of nutrient solution are similar