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Denitrifying sulfur conversion-associated EBPR: Effects of temperature and carbon source on anaerobic metabolism and performance

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ABSTRACT

The recently developed Denitrifying Sulfur conversion-associated Enhanced Biological Phosphorus Removal (DS-EBPR) process has demonstrated simultaneous removal of organics, nitrogen and phosphorus with minimal sludge production in the treatment of saline/brackish wastewater. Its performance, however, is sensitive to operating and environmental conditions. In this study, the effects of temperature (20, 25, 30 and 35 °C) and the ratio of influent acetate to propionate (100–0, 75–25, 50-50, 25–75 and 0–100%) on anaerobic metabolism were investigated, and their optimal values/controls for performance optimization were identified. A mature DS-EBPR sludge enriched with approximately 30% sulfate-reducing bacteria (SRB) and 33% sulfide-oxidizing bacteria (SOB) was used in this study. The anaerobic stoichiometry of this process was insensitive to temperature or changes in the carbon source. However, an increase in temperature from 20 to 35 °C accelerated the kinetic reactions of the functional bacteria (i.e. SRB and SOB) and raised the energy requirement for their anaerobic maintenance, while a moderate temperature (25–30 °C) resulted in better P removal (≥93%, 18.6 mg P/L removal from total 20 mg P/L in the influent) with a maximum sulfur conversion of approximately 16 mg S/L. These results indicate that the functional bacteria are likely to be mesophilic. When a mixed carbon source (75–25 and 50-50% acetate to propionate ratios) was supplied, DS-EBPR achieved a stable P removal (≥89%, 17.8 mg P/L for 400 mg COD/L in the influent) with sulfur conversions at around 23 mg S/L, suggesting the functional bacteria could effectively adapt to changes in acetate or propionate as the carbon source. The optimal temperatures or carbon source conditions maximized the functional bacteria competition against glycogen-accumulating organisms by favoring their activity and synergy. Therefore, the DS-EBPR process can be optimized by setting the temperature in the appropriate range (25–30 °C) and/or manipulating influent carbon sources.

1. Introduction

In densely populated cities where freshwater is scarce, using seawater or brackish water for sanitation can reduce fresh water consumption and save energy (Tang et al., 2007; Leung et al., 2012), but the saline sewage produced is rich in sulfate and must undergo treatment before it can be discharged into the sea. The conventional enhanced biological phosphorus removal (CEBPR) process that utilizes enriched polyphosphate-accumulating organisms (PAOs) (Wentzel et al., 1989; Kuba et al., 1993; Smolders et al., 1994) does

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not work well under high levels of sulfate (Yamamoto-Ikemoto et al., 1991; Baetens, 2001) and salinity (Welles et al., 2014). These conditions promote the overgrowth of unwelcome glycogen-accumulating organisms (GAOs), which compete against PAOs for the limited carbon sources and limit the application and performance of CEBPR in treating saline sulfate-rich sewage. The Denitrifying Sulfur conversion-associated Enhanced Biological Phosphorus Removal (DS-EBPR) process was subsequently developed for the removal of phosphorus (P) from saline/brackish wastewater in tropical and subtropical coastal regions such as Hong Kong. Saline sewage normally contains sulfate levels over 200 mg-S/L (Wu et al., 2016) and salt levels over 2% w/v NaCl (Bear et al., 1999; Xiao and Roberts, 2010).

In the DS-EBPR process, sulfate-reducing bacteria (SRB) (e.g. *Desulfobacter*, *Desulfobulbus*, *Desulfuromonas*) and sulfide-oxidizing bacteria (SOB) (e.g. *Thiohalomonas*, *Thiotrichaceae*, *Thiobacillus*) likely work synergistically to achieve P-release and uptake and compete against GAOs for carbon source (i.e. volatile fatty acids (VFAs)) (Guo et al., 2016a; Zhang et al., 2017). Specifically, under anaerobic conditions, SRB and SOB may cooperate in sulfate reduction to form polysulfide and/or elemental sulfur (poly-S) but they may simultaneously compete against each other for carbon source (Guo et al., 2016a; b; Zhang et al., 2017). Subsequently under anoxic conditions, SOB seemingly play the same role in DS-EBPR as conventional PAOs do in CEBPR by oxidizing intracellular poly-β-hydroxyalkanoates (PHA) and poly-S as the energy source for P uptake (Guo et al., 2016a; b). Indeed, Rubio-Rincón et al. (2017) recently found that *Thiothrix caldifontis*—a mixotrophic SOB—could store carbon anaerobically as PHA and use both PHA and poly-S to generate energy for aerobic phosphorus uptake in a CEBPR system. This novel EBPR process benefits from the sulfur (S) conversion involved (i.e. sulfate reduction and sulfide oxidation) due to lower energy yields (Wu et al., 2014). The process can simultaneously remove organics, nitrogen (N) and P with minimal sludge production (Guo et al., 2016b).

However, changes in operating or environmental conditions can lead to the overgrowth of GAOs, resulting in unstable system performance (Guo et al., 2017). Control strategies are needed to suppress the proliferation of GAOs and achieve stable/optimal DS-EBPR performance, but such strategies require an understanding of how operating or environmental conditions affect the competition between the functional bacteria (i.e. SRB and SOB) and the hostile GAOs. In biological P removal processes, certain operating and environmental conditions have been identified as key factors influencing the competition between PAOs and GAOs. These factors include pH, type of carbon source present in the influent (e.g. acetate (HAc) or propionate (HPr), the two most common VFAs in municipal wastewater (Oehmen et al., 2007)), temperature and the phosphorus-to-VFA ratio in the influent (Oehmen et al., 2007; Lopez-Vazquez et al., 2009). Although several primary operating and environmental factors such as nitrate concentration (Yu et al., 2016), salinity (Wu et al., 2014), sludge concentration, mixing intensity (Guo et al., 2016a; b), and pH (Guo et al., 2017) have been examined in the DS-EBPR process, its performance still fluctuated in previous studies (Wu et al., 2014; Guo et al., 2016a, b, 2017). Thus it is necessary to examine more comprehensively the optimal conditions for the functional bacteria and the disadvantageous for GAOs in DS-EBPR, such as temperature and carbon source, which have not been explored in DS-EBPR to date. Both temperature and carbon source vary diurnally and seasonally in practical wastewater treatment processes. Such undesired fluctuations may adversely affect the functional bacteria of the DS-EBPR process, including their ability to metabolize S, N and P. Moreover, studying the anaerobic metabolism of the functional bacteria and GAOs under different temperature and carbon source conditions is an effective way to understanding their competition (Oehmen et al., 2005; Lopez-Vazquez et al., 2007).

This study therefore aims to investigate the short-term effects of temperature and the ratio of HAc to HPr on DS-EBPR. The temperatures investigated were 20, 25, 30 and 35 °C while the ratios of HAc to HPr tested, determined on the basis of mg TOC/L of influent, were 100–0, 75–25, 50-50, 25–75 and 0–100%. The stoichiometry and kinetics, which reflect the anaerobic metabolism, and the reactor performance in terms of P removal and S conversion were evaluated. The results are compared with those on the behavior of enriched PAOs, GAOs and S bacterial cultures published previously. Finally, strategies based on manipulating the temperature and the carbon source are proposed for optimizing this technology.

2. Materials and methods

2.1. Continuous operation of the sequencing batch reactor

A tightly-sealed lab-scale sequencing batch reactor (SBR) with a working volume of 20 L was used to enrich the functional bacteria of DS-EBPR, i.e. SRB and SOB. The reactor had been continuously operated for over 500 days, with approximately 9.6 g total suspended solid (TSS)/L of sludge and a ratio of mixed liquor volatile suspended solids (MLVSS) to TSS of 0.72 as detailed in our previous work (Guo et al., 2017). The SBR was operated cyclically under alternating anaerobic and anoxic conditions. The durations of the anaerobic and anoxic phases were varied to allow the target metabolic reactions to be completed, such as P release/uptake, carbon source uptake and nitrate consumption (Wu et al., 2013). The reactor temperature was controlled at 30 ± 1 °C using a water bath to mimic the typical sewage temperature in Hong Kong and the pH was maintained between 7.2 and 7.9 by adding a 0.5 M HCl solution and/or a 0.5 M NaOH solution as necessary using a pH controller. The sludge retention time (SRT) was maintained at about 30 days by periodically withdrawing approximately 6.4 g TSS/d of sludge which includes TSS loss via the effluent and routine sampling. The total phosphorus content in MLVSS and an average P mass balance analysis are shown in the Supplementary Information (SI).

Synthetic saline sewage containing 20% seawater and 80% freshwater (~0.7% salinity in the mixture) was prepared to simulate the influent carbon-to-sulfur (C/S) ratio of typical saline sewage in Hong Kong (approximately 1.0 mg C/mg S), as described in Guo et al. (2016a). The synthetic saline sewage was composed of 60 mg of NH_4^+-N/L, 20 mg of PO_4^{3-}-P/L, 267 mg of HAc as chemical oxygen demand (COD)/L (equivalent to 100 mg TOC/L), and 133 mg of HPr as COD/L (equivalent to 50 mg TOC/L), resulting in a ratio of acetate to propionate of 67–33% in the influent to simulate the common conditions in wastewater (Oehmen et al., 2007). This gave a total concentration of organics of 400 mg COD/L, equivalent to 150 mg TOC/L and 150-200 mg-S/L of sulfate on average. The influent exchange volume ratio was 0.5.

2.2. Batch experiments

A test was carried out in duplicate to examine the cyclic behavior of the functional bacteria in the DS-EBPR SBR on day 455 (see Guo et al., 2017 for details). At the end of the anoxic phase on day 465 and day 470, 2000 and 2500 mL of mixed DS-EBPR sludge were taken from the SBR for short-term temperature and carbon source batch tests respectively (see Table 1). The sludge was prepared as detailed in Guo et al. (2017). The sludge mixed liquor taken from the SBR was evenly transferred into four or five 500 mL batch reactors and mixed in an orbital shaker. Then 250 mL of the same synthetic saline wastewater as described in Section 2.1 above were