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A new biological phosphorus removal process in association with sulfur cycle

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ARTICLE INFO

Article history:
Received 7 November 2012
Received in revised form
11 February 2013
Accepted 5 March 2013
Available online 27 March 2013

Keywords:
SANI process
Saline sewage
Enhanced biological phosphorus removal (EBPR)
Sulfur cycle

ABSTRACT

Hong Kong has practiced seawater toilet flushing since 1958, saving 750,000 m^3 freshwater every day. A high sulfate-to-COD ratio (>1.25 mg SO_4/mg COD) in the saline sewage resulting from this practice has enabled us to develop the Sulfate reduction Autotrophic denitrification and Nitrification Integrated (SANI®) process with minimal sludge production. This study seeks to expand the SANI process into an enhanced biological phosphorus removal (EBPR) process. A sulfur cycle associated EBPR was explored in an alternating anaerobic/oxygen-limited aerobic sequencing batch reactor with acetate fed as sole electron donor and sulfate as sulfur source at a total organic carbon to sulfur ratio of 1.1–3.1 (mg C/mg S). Phosphate uptake and poly-phosphate formation was observed in this reactor that sustained high phosphate removal (20 mg P/L removed with 320 mg COD/L). This new EBPR process was supported by six observations: 1) anaerobic phosphate release associated with acetate uptake, poly-phosphate hydrolysis, poly-hydroxyalkanoate (PHA) (and poly-S^{2-}/S^0) formation and an "aerobic" phosphate uptake associated with PHA (and poly-S^{2-}/S^0) degradation, and polyphosphate formation; 2) a high P/VSS ratio (>0.16 mg P/mg VSS) and an associated low VSS/TSS ratio (0.75) characteristic of conventional PAOs; 3) a lack of P-release and P-uptake with formaldehyde inactivation and autoclaved sterilized biomass; 4) an absence of chemical precipitated P crystals as determined by XRD analysis; 5) a sludge P of more than 90% polyphosphate as determined by sequential P extraction; and 6) microscopically, observed PHA, poly-P and S globules in the biomass.

1. Introduction

Water scarcity is affecting many densely populated cities, particularly in the developing world. The situation is even worsening as a result of population growth, climate change and uneven precipitation (Bates et al., 2008; WHO, 2010). To alleviate water shortage, Hong Kong has been using seawater for toilet flushing since 1958. The system is now

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http://dx.doi.org/10.1016/j.watres.2013.03.009

covering 80% of its 7 million inhabitants and saving 750,000 m^3 of freshwater every day (Tang et al., 2007; Leung et al., 2012). More applications in the direct use of seawater as an alternative resource are being developed, e.g. cooling systems and urine phosphorus (P) recovery (van Loosdrecht et al., 2012). One of these applications makes use of the sulfate originating from seawater toilet flushing to serve as an electron carrier to realize a new sulfur-based biological nitrogen removal process for saline wastewater, namely the Sulfate reduction Autotrophic denitrification Nitrification Integrated (SANI®) process (Lau et al., 2006; Wang et al., 2009).

The SANI process modifies the conventional biological nitrogen removal process by adding an anaerobic reactor at the beginning, utilizing sulfate as the electron acceptor. In this reactor, sulfate (a strong acid) is reduced to sulfide (a weak acid), therefore the pH will rise maintaining the produced sulfide in the dissolved form. Due to a surplus of sulfate relative to COD, no methanogenesis will occur. Sulfide acts in the next step as the electron donor for autotrophic denitrification in the anoxic reactor where it is oxidized back to sulfate, completing the sulfur cycle. The three major microbial groups, sulfate-reducing bacteria (SRB), sulfide-oxidizing bacteria (SOB) and nitrifiers, all have a low growth yield (Comeau, 2008), therefore the sludge yield in the SANI process is minimized. Compared with conventional biological treatment with the excess sludge disposed by incineration, the SANI process can save one third of the energy consumption and CO_2 emission (Lu et al., 2012a,b).

For full nutrient removal, integration of the biological phosphate removal in the SANI process would be desired. Enhanced Biological Phosphorus Removal (EBPR) process is well established and is based on aerobic or denitrifying polyphosphate accumulating organisms (PAOs) (Wentzel et al., 1988; Jenkins and Tandoi, 1991; van Loosdrecht et al., 1997; Mino et al., 1998; Oehmen et al., 2007). If PAOs can be cultivated in association with the sulfur cycle, the SANI process could include the EBPR process. However, it has been suggested that conventional PAOs could be hindered by sulfate reduction (Yamamoto-Ikemoto et al., 1991, 1994; Baetens, 2000).

To investigate and develop a sulfur associated EBPR bioprocess, a lab-scale sequencing batch reactor (SBR) was operated based on an anaerobic/anoxic cycle operation (Kuba et al., 1993), but using sulfate as the electron acceptor in an early study (Wu et al. 2012). While this study did observe some potential signs of possible biological P-release and P-uptake at high sulfate levels, the electron acceptor was unclear and the mechanism was not well demonstrated. In this paper, the laboratory-scale system was continued and the research directions focused on: (i) confirming the phenomenon as a biological process through inactivating and sterilizing the sludge and observing the biomass poly-phosphate accumulation through microscopic examinations; (ii) determining the electron acceptors of this sulfur associated biological P-release/-uptake phenomenon; (iii) assessing the impact of sulfate increase on the P-release/-uptake; and (iv) examining the sulfur associated biological P removal processes for possibly upgrading the existing SANI process to include an EBPR process.

2. Materials and methods

2.1. Reactor design, wastewater composition and operation conditions

A lab-scale Sequencing Batch Reactor (SBR) was made of opaque PVC, having a total reactor volume of 30 L (20 L reaction volume and 10 L headspace), as shown in supplementary Fig. 1a. The reactor was tightly sealed and continuously operated for 280 days under mixing (using a mechanical mixer, at 150 rpm). The reactor temperature was controlled at $22 \pm 1\,^{\circ}C$. During the first 60 days of operation, as detailed in our previous paper (Wu et al. 2012), the reactor pH was controlled at 7.4–7.9, but for the last 220 days of operation, pH control was not necessary as the reactor pH was fairly constant. The inoculum of the reactor was taken from the anaerobic digestor of a local saline sewage treatment works, Sha Tin Swewage Treatment Works (STSTW) in Hong Kong, which contains sufficient sulfate reducing bacteria (Ye and Zhang, 2012).

Synthetic wastewater followed the modified composition of Kuba et al. (1993), and the composition was described in Wu et al. (2012). The only organic substrate was sodium acetate. Sulfate was provided by dosing with a sodium sulfate solution in the SBR to meet the designed influent carbon: sulfur ($C_{inf.}/S_{inf.}$) ratio. Salinity inhibition on P removal in freshwater wastewater EBPR was observed in the presence of 0.5% salt content (Uygur and Kargi, 2004). However, saline sewage that contained up to 1% salinity did not impact the SANI process significantly during its pilot trial (Lu et al., 2012b). This certainly does not deny possibility of salts impact on the EBPR. In this initial exploratory investigation of sulfur cycle effect on EBPR, salinity was not introduced in order to confirm sulfur cycle associated EBPR first. A future study on possible impact of salt content on this novel EBPR will be conducted.

Supplementary Fig. 1b illustrates the cyclic operation of the SBR, including: (I) feeding of 10 L of synthetic wastewater (in 15 min), (II) reaction phase R, i.e. P-release phase, (III) addition of sulfate, (IV) reaction phase U, i.e. P-uptake phase, (V) 1 h settling, and (VI) decantation of 10 L of supernatant (in 15 min). In each cycle, around 4 to 10 samples at a volume of 2 or 20 mL were periodically taken from the reactor in order to monitor the SBR performance. The 2-mL samples were first filtered with 0.22-μm filter for analyzing anions of acetate, chlorate, nitrate, nitrite, phosphate, sulfate and thiosulfate. These measurements were used to set the time allowed for the two reaction phases R and U to ensure completion of the P-release and P-uptake reactions before moving to the next phase, i.e. for acetate to reach zero and the bulk phosphate level to decrease in reaction phase R and for the phosphate concentration to stabilize in reaction phase U. The SBR cycle time was therefore controlled by the P-release/-uptake bioprocess rates. The SBR cycle times decreased as the P-release/-uptake rates increased during the investigation. The 20 mL samples were used for analysis of the mixed liquor suspended solids (TSS), mixed liquor volatile suspended solids (VSS), total phosphorus (TP), and sulfide.

EBPR functions successfully in freshwater-based sewage with a total organic carbon to SO_4^{2-}–S ratio of 16 (mg C/mg S) (Tchobanoglous et al., 2004), correspondingly, the possibility of