Bioresource Technology 171 (2014) 80–87

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

The effect of COD loading on the granule-based enhanced biological phosphorus removal system and the recoverability

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HIGHLIGHTS

- The threshold of COD shock loading for metabolism of PAOs is 500 mg L^{-1}.
- COD beyond 600 mg L^{-1} would deteriorate granular EBPR system irreversibly.
- High COD loading could inhibit the excretion of EPS, especially polysaccharides.
- High COD loading changed the transformation patterns of PHAs and glycogen.
- High COD loading may provide a competitive advantage to GAOs over PAOs.

ARTICLE INFO

Article history:
Received 23 June 2014
Received in revised form 9 August 2014
Accepted 11 August 2014
Available online 20 August 2014

Keywords:
Granule-based EBPR system
COD loading
Extracellular polymeric substances
Metabolism process of PAOs
Recoverability

ABSTRACT

In this study, the effect of varied COD loading (200, 400, 500, 600 and 800 mg L^{-1}) on stability and recoverability of granule-based enhanced biological phosphorus removal (EBPR) system was investigated during continuously 53-d operation. Results showed that COD loading higher than 500 mg L^{-1} could obviously deteriorate the granular EBPR system and result in sludge bulking with filamentous bacteria. High COD loading also changed the transformation patterns of poly-β-hydroxyalkanoates (PHAs) and glycogen in metabolism process of polyphosphate-accumulating organisms (PAOs) and inhibited the EPS secretion, which completely destroyed the stability and integrality of granules. Results of FISH indicated that glycogen-accumulating organisms (GAOs) and other microorganisms had a competitive advantage over PAOs with higher COD loading. The community composition and EBPR performance were recovered irreversibly in long time operation when COD loading was higher than 500 mg L^{-1}.

1. Introduction

Phosphorus (P), which was considered as one of key factors to accelerate eutrophication and deteriorate water quality, has been paid more attention to, and stimulated research and development of biological phosphorus removal process. The enhanced biological phosphorus removal (EBPR) process is becoming a popular method for P removal from wastewater because of its relatively low cost and environmentally sustainability (Mielczarek et al., 2013). With alternating anaerobic and aerobic conditions, successful operation of EBPR process relies on the ability of polyphosphate-accumulating organisms (PAOs) to store P as polyphosphate inside the cells. And it can achieve P removal through discharging surplus sludge appropriately (Oehmen et al., 2007; McMahon et al., 2010). Particularly, due to the integration of biogranulation technologies, the granule-based EBPR process could achieve better performance as a result of high biomass level, excellent settling ability, and ability to withstand high organic loading and endure to toxicity (Liu et al., 2003; Wang et al., 2007).

During past few years, effects of carbon resource and the substrate P/COD ratio on EBPR system have been studied widely. Guerrero et al. (2011) found that when the mix of propionic acid, acetic acid and sucrose were used as influent COD ingredients, the EBPR was maintained surprisingly. However, it resulted in a fast failure system when sucrose was used as single carbon source. Moreover, using glycerol as an external carbon source could increase P removal efficiency in dairy wastewater treatment (Guerrero et al., 2012). The carbon source could also strongly influence both anabolic (growth, P-uptake and glycogen production)

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http://dx.doi.org/10.1016/j.biortech.2014.08.057
0960-8524/

and catabolic (poly-hydroxyalkanoates oxidation) processes of the Accumulibacter PAOs to different extents: Puig et al. (2008) pointed out that the poly-β-hydroxyalkanoate (PHA) of PAOs became poly-β-hydroxyvalerate (PHV) (81.9%) through 140 d of ethanol acclimatizing and the phosphate concentration in the effluent was close to zero. Furthermore, different COD/P ratios and various kinds of carbon resource could affect PAOs in their competition with glycogen-accumulating organisms (GAOs) in EBPR systems. Panswad et al. (2007) indicated that a lower COD/P feeding ratio could obviously promote the growth of PAOs and a dynamic situation of the microbial population. Broughton et al. (2008) carried out various COD/P ratios experiments and found that GAOs lost out to PAOs when grown in the absence of the varying carbon. Additionally, propionate was more profitable than acetate for PAOs in the continuous plug-flow anaerobic/aerobic (A/O) process while glucose worsened the EBPR performance through giving GAOs a metabolic competitive advantage over PAOs (Wang et al., 2010).

Nevertheless, most of the above studies were mainly focused on the traditional enhanced biological phosphorus removal system, which was quite different with the granule-based EBPR system. The formation and stability of granular sludge were affected by hydraulic condition, aeration intensity, carbon resource, COD loading, nitrite, free ammonia (FA) and metal ions, etc (Oehmen et al., 2007; Zheng et al., 2013b; Fang et al., 2012). However, few researchers penetrated into the long-term effect of COD shock loading on the EBPR granular sludge from macro to micro-scale or the recoverability of PAOs granules after COD shock loading. Urban sewage in China is normally mixed with industrial wastewater. As a result, the quality, especially COD concentration of wastewater fluctuates dramatically. Since EBPR systems are widely studied and applied, it is necessary to take in-depth research to recognize the effect mechanism of COD loading on phosphorus removal granules. Therefore, the main objective of this study is to investigate the effect of COD loading on the stability and recoverability of granule-based EBPR system at different COD levels. Phosphate and COD removal performance, characteristics and morphology of the granules, extracellular polymeric substances (EPS), metabolism of PHAs and glycogen and PAOs/GAOs competition with synthetic wastewater were investigated to evaluate the stability of the system. Meanwhile, fluorescence in situ hybridization (FISH) was applied for sake of characterizing the microbial community structure in bioreactors. After EBPR performance became completely deteriorated, the recoverability was evaluated to judge if the system was reversible. Meanwhile, the recoverability of PAOs performances was also analyzed which has certain significance in practice.

2. Methods

2.1. Cultivation of EBPR granules

The EBPR granules were cultivated in two sequencing batch reactors (SBR) with working volume 10 L, performing four cycles of alternating anaerobic–aerobic conditions every day. Each cycle lasted for 6 h, involving 5 min feeding of 2.5 L synthetic wastewater, 2.5 h of anaerobic stirring, 3 h of aerobic reaction, 5 min of precipitation, 5 min of effluent discharge, and 15 min of idling. In order to provide PAOs further competitive advantages over GAOs, acetate and propionate are applied as carbon source in turn every 10 d (Lu et al., 2006; Zheng et al., 2013b). High enrichment of PAOs was obtained after 75 d of acclimation. Then steady phosphorus and COD removal performance was obtained in the granule-based EBPR system through three months of operation. Thus the mature granular sludge was prepared when the PAOs became the predominant bacteria by FISH analysis and the mixed liquor suspended solids (MLSS) (granular sludge concentration) reached 2500 mg L^{-1}.

2.2. Synthetic wastewater

The composition of the synthetic wastewater was as follows (per litter): 0.256 g of CH_3COONa; 0.4 ml of CH_3CH_2COOH; 0.2293 g of NH_4Cl; 0.0875 g of KH_2PO_4; 0.147 g of $K_2HPO_4{\cdot}3H_2O$; 0.1845 g of $MgSO_4{\cdot}7H_2O$; 0.0222 g of $CaCl_2$; 0.0015 g of peptone; 0.0015 g of yeast extract powder; 0.0012 g of allylthiourea (ATU); 0.6 ml of trace elements solution. The composition of trace element solution referred to Smolders et al. (1994). Propionate and acetate in the ratio of 3/1 were used to provide carbon source, while NH_4Cl was used to provide N source, and KH_2PO_4 and K_2HPO_4 were used to provide P sources. Besides, ATU was used to inhibit nitrification (Liu et al., 2011; Zheng et al., 2013a). In the enrichment phase of PAOs, synthetic medium were fed into each SBR to reach 200 mg L^{-1} COD, 10 mg $L^{-1}PO_4^{3-}$-P and 15 mg L^{-1} NH_4^+-N at the beginning of the anaerobic phase of each cycle.

2.3. Loading experiment at different COD concentration

After the enrichment of PAOs, different amount of acetate and propionate were added into four parallel SBRs, in order to obtain initial COD concentrations of 200 mg L^{-1} (R1), 400 mg L^{-1} (R2), 600 mg L^{-1} (R3) and 800 mg L^{-1} (R4). During loading experiment, quick break-down were observed in R3 and R4, which made it difficult to estimate the threshold of COD shock loading for granular EBPR system. Therefore, another SBR (R5) with COD concentration of 500 mg L^{-1} was added on Day 8. Granules in each SBR were cultivated under the same operation condition to maintain MLSS concentration around 2500 mg L^{-1}. Excess sludge was discharged through the measurement of MLSS every day calculated by the equation: $V_{discharge} = V-(2500\ mg\ L^{-1} * V/m_{MLSS})$, ($V_{discharge}$ = the sludge discharged, V = the working volume of SBR, m_{MLSS} = the quality of the measurement about MLSS every day). Sludge retention time (SRT) was about 7 d according to the discharge of excess sludge and hydraulic retention time (HRT) was 24 h. The temperature was controlled at 20 ± 2 °C by air conditioning. The pH of each system was adjusted to 7.0 ± 0.1 by adding 1 M NaOH, and dissolved oxygen (DO) in aerobic was controlled in the range of 6.0–7.0 mg L^{-1}.

The experiment included two phases: the test of resistance to COD loading (34 d) and the test of recoverability of break-down system (19 d). R1 was served as a reference while R2, R3, R4 and R5 were applied as test groups to attain the threshold value of COD shock loading. After 34-d operation, when the P removal ability of granular EBPR system was completely destroyed, loading experiment was stopped and the COD concentration in each reactor was recovered to 200 mg L^{-1}. The characteristic of sludge was investigated if COD loading was reversible. The recovery test lasted for 19 d until the SBRs reached a steady performance (keeping for at least one week).

2.4. Chemical analysis

Samples were collected at the feeding stage, the end of anaerobic stage and aerobic stage, respectively. They were immediately filtered through disposable qualitative filter paper (0.22 μm pore size) before analyzing. Parameters including COD, phosphate (PO_4^{3-}-P), MLSS, mixed liquor volatile suspended solids (MLVSS), sludge volumetric index (SVI) and sludge volumetric in 30 min (SV_{30}) were analyzed by standard methods (APHA, 1998).

Extracellular polymeric substances (EPS) were extracted and analyzed referring to Wang et al. (2006). Amount of EPS was