Effect of Nitrite and External Carbon Source on the Via Nitrite Biological Phosphorus Removal

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The via nitrite biological phosphorus removal was investigated in batch experiments using biomass collected from a sequencing batch reactor (SBR) treating low strength nitrogenous effluents. The effect of the initial nitrite concentration and the external carbon source characteristics on the via nitrite specific phosphorus uptake rate (sPUR) was investigated. The external carbon sources that were applied consisted of fermented liquid produced from the organic fraction of municipal solid waste (OFMSW FL), fermented liquid produced from vegetable and fruit waste (VFW FL) and VFW enriched with propionic acid and/or butyric acid. The type and composition of the applied external carbon source critically impacted the via nitrite sPUR. The addition of OFMSW FL resulted in very high via nitrite sPUR (7.25 mgP/(gVSS·h)) and specific phosphorus release rates (sPRR=5.11 mgP/(gVSS·h)). The presence of propionic acid and butyric acid in OFMSW FL promoted phosphorus uptake. The short chain fatty acids (SCFAs) in the VFW FL consisted mainly of acetic acid and resulted in lower sPUR. Furthermore, increased concentration of SCFAs in the mixed liquor enhanced the via nitrite phosphorus removal. The presence of nitrite in the mixed liquor did not adversely impact the phosphorus uptake mechanism up to the concentration range of 50-70 mg/L. However, higher nitrite levels (100-120 mg/L) resulted in some sPUR inhibition.

1. Introduction

Denitrifying via nitrite biological phosphorus removal (DNBPR) offers the possibility to integrate phosphorus and nitrogen removal; in the presence of nitrite and the lack of oxygen, nitrite is denitrified to gaseous nitrogen and simultaneously the phosphate is taken by denitrifying phosphorus accumulating organisms (DPAOs) (Peng et al., 2011). DPAOs are able to accumulate significant amounts of polyphosphate, as the phosphorus accumulating organisms (PAOs) do in the conventional enhanced biological phosphorus removal process. This results from the ability of DPAOs to store significant amounts of polyphosphate under anoxic conditions. DPAOs are approximately 40% less efficient in terms of energy generation than PAOs and have 20-30% lower cell yield (Murnleitner et al., 1997).

The enhanced biological phosphorus removal (EBPR) process requires a significant concentration of short chain fatty acids (SCFAs), which are taken up by PAOs to form polyhydroxyalkanoates (PHAs) during anaerobic conditions. Often the organic carbon source provided by the effluents is not sufficient (particularly when anaerobic effluents are treated) and an external organic carbon source must be supplied to enhance the nutrient removal. The addition of commercially available SCFAs increases the operating cost of the process, particularly since the price of the commonly used synthetic carbon sources has increased significantly over the last years. The use of waste derived carbon sources can render the process economically more favourable. Apart from the SCFA concentration, their composition can also affect the performance of EBPR (Feng et al., 2009). Recent works have shown that the carbon sources that contain a mixture of SCFAs can improve DNBPR (Ji and Chen, 2010; Frison et al., 2103a). Frison et al. (2013a; 2013b) and Fatone et al. (2011) achieved significant via nitrite phosphorus (>80%) and nitrogen (>85%) removal from the anaerobic supernatant resulting from the co-digestion of the organic fraction of municipal solid waste (OFMSW) and waste activated sludge. Frison et al. (2013a; 2013c) employed three different types of organic carbon source which included the OFMSW fermentation liquid (FL), drainage liquid from OFMSW and FL from cattle manure and maize silage. In batch reactors, the OFMSW FL

resulted in the highest rate of phosphate uptake via nitrite, while high rates were also obtained with the FL from cattle manure and maize silage. Ji and Chen (2010) achieved 97.6% of soluble phosphorus removal from low strength effluents by DNBPR using sludge fermentation liquid as an external carbon source, while the use of acetic acid resulted only in 73.4%. A potential problem in the DNBPR is that the exposure to high nitrite concentrations may inhibit the activity of DPAOs. Several threshold nitrite values have been reported in the literature ranging from 3-93.7 $mgNO_2$-N/L (Meinhold et al., 1999; Saito et al., 2004; Zhou et al., 2007; Peng et al.2011).

2. Materials and methods

2.1 Collected Biomass
An upflow anaerobic sludge blanket (UASB) treated synthetic domestic wastewater. The UASB effluent then in turn was treated from a sequencing batch reactor (SBR) having a reaction volume of 26 L. The UASB effluent was characterized by a very low chemical oxygen demand to nitrogen ratio (COD/N<2). The biomass was collected during the anoxic period of operation when the dissolved oxygen (DO) level was below 0.05 mg/L in order to be used for the ex situ activity experiments.

2.2 Biomass activity experiments
In all biomass activity experiments, 300 mL of biomass was placed in 1 L Erlenmeyer flasks under mild agitation, the temperature was maintained at 25±2°C and the pH was controlled at 7.4±0.2. The biomass was spiked with specific (depending on the experiment) nitrite and phosphate (i.e. 30 mg PO_4-P/L) concentration as well as with an external organic carbon source. The top part of the flask was covered with aluminium foil to prevent air ingress. Then, the variation of the nitrite and phosphate concentration with time was monitored in order to determine the specific nitrite uptake rate (sNUR) and the specific phosphate uptake rate (sPUR). In the specific phosphate release rate (sPRR) experiments the biomass was collected at the end of the anoxic phase of the SBR operation and was placed under mild agitation in closed vials; argon was supplied for 3 min to ensure the anaerobic environment. An analysis of the NO_x-N was conducted to ensure nitrite and nitrates were zero. Then, the biomass was spiked with external carbon source and the release of phosphorus with time was recorded.

The OFMSW FL and VFW FL were produced in the laboratory through the controlled acidogenic fermentation of OFMSW and VFW (temperature = 37°C for OFMSW and 30°C for VFW, organic loading rate = 20 $kgTVSm^{-3}d^{-1}$, hydraulic retention time = 5d, pH = 4.1-4.5). The sNUR, sPUR and sPRR values that are reported have been corrected to the reference temperature of 20°C using the Arrhenius temperature correction factor and have been normalized to the volatile suspended solids (VSS) concentration of the biomass. In certain experiments the concentration of VFW FL was increased to determine the effect of the sCOD on the phosphorus uptake and release rates. Furthermore, a complete reaction SBR cycle was simulated by placing the biomass first under anaerobic conditions, adding ammonium (26.2, 43.9 and 79.6 $mgNH_4$-N/L) and excess OFMSW FL. The biomass was maintained for 20 min in the anaerobic phase, 100 min in aerobic phase (continuous aeration with DO > 4 mg/L) and then in the 40 min anoxic phase; the nitrite and phosphate concentrations in the mixed liquor were determined.

2.3 Analytical methods
Total suspended solids (TSS), VSS, pH, chemical oxygen demand (COD), ammonium nitrogen (NH_4-N), total Kjeldahl nitrogen (TKN), nitrite (NO_2-N) and nitrate (NO_3-N), phosphorus (P) and phosphate (PO_4-P) were determined according to standard methods (APHA, AWWA, WEF, 1998) and ion chromatography (Dionex ICS-90 with AG14 and AS14 columns) for the anions. The soluble COD (sCOD) was determined in permeate collected after the filtration through Whatman 0.45 µm membrane filters. Acetic acid (HAc), propionic acid (HPr) and butyric acid (HBut) were analyzed by gas chromatography (Column: Nukol 15 m, 0.53 ID; temperature: 85-125°C, 30°C/min; carrier: N_2, 5 mL/min).

3. Results and discussion

3.1 External carbon sources
Table 1a summarizes the physicochemical characteristics of the OFMSW FL and the VFW FL and Table 1b shows the SCFAs contained in the two carbon sources and in the VFW FL that was then enriched with butyric acid and propionic acid. The OFMSW FL was characterized by much higher concentration of sCOD than VFW FL. More importantly, the OFMSW was rich not only in acetic acid but also in butyric and propionic acid. Both fermentation liquids and particularly the OFMSW FL contained high concentrations of organic nitrogen and ammonium and phosphate. The fermentation process resulted in the hydrolysis of particulate organic nitrogen to soluble TKN, the ammonification of TKN to ammonium and the hydrolysis of organic phosphorus to phosphate. The presence of high nitrogen and phosphorus in the waste derived carbon source is clearly a disadvantage since it increases the nutrients load that must be removed in the SBR process.

Table 1a Physicochemical characteristics of the external carbon sources

Parameter	OFMSW FL	VFW FL
pH	4.22 ± 0.21	4.35 ± 0.17
COD (mg/L)	45009 ± 14288	22858 ± 5346
sCOD (mg/L)	34789 ± 14549	11958 ± 2045
TKN (mg/L)	1150 ± 330	408 ± 274
NH_4-N (mg/L)	337 ± 122	130 ± 108
P (mg/L)	156 ± 45	49 ± 25
PO_4-P (mg/L)	138 ± 80	37 ± 17

Table 1b VFAs contained in the external carbon sources before and after the enrichment with SCFAs

Acid	OFMSW FL	VFW FL	VFW FL + HBut	VFW FL + HPr	VFW FL + HPr + HBut
Acetic acid (mg/L)	5721 ± 493	2279 ± 212	2033 ± 176	2210 ± 265	2039 ± 182
Propionic acid (mg/L)	2342 ± 192	5.2 ± 3.8	26.0 ± 5.9	1048 ± 109	1102 ± 78
Butyric acid (mg/L)	5021 ± 200	88.8 ± 13.9	1567 ±78	197 ± 22	1746 ± 113

3.2 Effect on nitrite concentration

The effect of the initial nitrite concentration on the via nitrite sPUR was investigated. The increase of nitrite concentration from 32.5 $mgNO_2$-N/L to 66.1 $mgNO_2$-N/L did not adversely impact on the via nitrite sPUR, showing that DPAOs could tolerate such nitrite levels (Table 2a). The further increase of the initial nitrite concentration to 99.2 $mgNO_2$-N/L resulted in a decrease of sPUR by 17% and 24% compared to the low and medium nitrite levels respectively. On the contrary, the denitritation rate was not adversely affected by the high nitrite levels. A very similar trend was obtained in Table 2b since the increase of initial nitrite up to 52.9 $mgNO_2$-N/L did not inhibit sPUR, while the further increase to 119.4 $mgNO_2$-N/L resulted in an inhibition of sPUR by 36% and 28% compared to the low and medium nitrite levels respectively. The sPUR values of Table 2b are higher than the respective values of Table 1a because the VFW FL was enriched with propionic acid and butyric acid.

Table 2a Effect of initial nitrite concentration on the sNUR, sPUR and sPRR using VFW FL as external carbon source

Initial NO_2-N mg/L	sNUR 20°C $mgNO_2$-N/(gVSS·h)	sPUR 20°C mgP/(gVSS·h)	sPRR 20°C mgP/(gVSS·h)
32.5	2.44	2.22	2.24
66.1	3.17	2.41	3.79
99.2	3.60	1.84	2.61

Table 2b Effect of initial nitrite concentration on the sNUR, sPUR and sPRR using VFW FL enriched with propionic and butyric acid as external carbon source

Initial NO_2-N mg/L	sNUR 20°C $mgNO_2$-N/(gVSS·h)	sPUR 20°C mgP/(gVSS·h)	sPRR 20°C mgP/(gVSS·h)
26.0	3.24	4.64	2.07
52.9	3.51	4.14	2.46
119.4	4.08	2.97	2.27

A complete SBR reaction operation was simulated in ex situ experiments for three different initial ammonium concentrations that were spiked at the initiation of the anaerobic phase (Figure 1). As a result, the nitrite levels reached a maximum of 19.4, 41.0 and 59.9 $mgNO_2$-N/L at the end of the aerobic phase for the three different experiments. However, these nitrite levels did not inhibit the subsequent via nitrite sPUR since high phosphorus removal rates were obtained on the subsequent anoxic conditions in all cases.
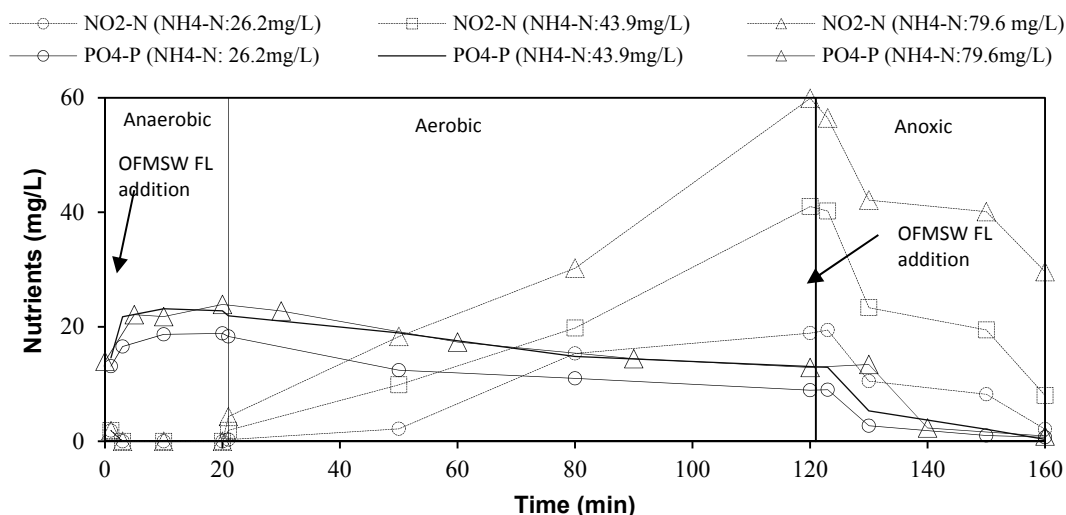
Figure 1 Profile of nitrite and phosphate ex situ simulation of the SBR reaction phases

3.3   Effect of carbon source type and concentration

The effect of the type and concentration of the applied external organic carbon source to the anoxic sPUR was investigated. As seen in Table 3a, the OFMSW FL resulted in much higher anoxic sPUR and anaerobic sPRR compared to the VFW FL. The latter carbon source contained acetic acid as SCFAs and negligible amounts of propionic acid and butyric acid (Table 1b). However, even its acetic acid concentration was much lower than that of OFMSW FL (2.3 g/L compared to 5.7 g/L). The enrichment of VFW with propionic acid and butyric acid (the concentrations are shown in Table 1b) resulted in a significant increase of sPUR and sPRR (Table 3a). However, the sPUR was still much lower than the rate obtained using OFMSW FL. The latter had higher concentration of these acids. These results showed that both the type as well as the concentration of SCFAs impact on the via nitrite sPUR.

To demonstrate further the importance of the SCFAs concentration on the via nitrite phosphorus uptake, different concentrations of VFW FL were added and the sNUR, sPUR and sPRR were measured. The increase of sCOD in the reactor resulted in an increase of the via nitrite phosphorus removal confirming that the concentration of SCFAs in the mixed liquor is important and affects the phosphorus uptake rate (Table 3b).

Table 3a Effect of external carbon source composition on sNUR, sPUR and PRR

Type of external carbon source	sPUR 20°C mgP/(gVSS·h)	sPUR 20°C mgP/(gVSS·h)	sPRR 20°C mgP/(gVSS·h)
OFMSW FL	7.25	7.25	5.11
VFW FL	1.01	1.01	1.31
VFW FL + HBut	2.28	2.28	2.02
VFW FL + HPr	2.77	2.77	2.54
VFW FL + HPr + HBut	4.18	4.18	4.21

Table 3b Effect of the sCOD concentration

Initial sCOD mg/L	sPUR 20°C mgP/(gVSS·h)	sPRR 20°C mgP/(gVSS·h)
231	2.91	2.19
401	3.83	3.06
756	4.93	4.86

3.4 Effect of electron acceptor

The results of previous studies have demonstrated that DPAOs acclimated to nitrite are capable of using nitrite as sole electron acceptor to perform denitritation and phosphorus removal even when nitrite levels are significant (Guisasola et al., 2009; Vargas et al., 2011). There are two groups of DPAOs (Hu et al., 2003): the over nitrate DPAOs that are able to use both oxygen and nitrate as electron acceptor and the DPAOs over nitrite group that are able to use oxygen, nitrate and nitrite as electron acceptor. It has been

postulated that DPAOs are able to reduce nitrate, while non-DPAOs (or simply, PAOs) are unable to reduce nitrate but able to reduce nitrite (Carvalho et al., 2007; Oehmen et al., 2010).

An investigation of the effect of electron acceptor (NO_2-N and NO_3-N) was conducted by performing batch tests under anoxic conditions with denitrification running over nitrite or nitrate. VFW FL enriched with propionic acid and butyric acid was used as external carbon source in all cases. The aim was to identify the effect of complete denitrification and denitritation on the rates of phosphorus removal. As shown in Table 4 the rate of NO_2-N reduction was significantly higher compared to the respective one of NO_3-N, while the via nitrite pathway resulted in higher rates for phosphorus uptake and release. Significantly higher rates of nitrogen and phosphate uptake were obtained by Guisasola et al. (2009) in the presence of NO_2-N instead of NO_3-N as electron acceptor using propionic acid as carbon source in both cases. Also, the authors reported the nitrite-DPAO failure to use nitrate as electron acceptor after some period of periodic nitrate feeding.

Table 4 Nutrient removal rates in NO_2-N and NO_3-N environment (average values ± standard deviations)

Electron acceptor	sNUR (mgNO₂-N/gVSS·h)	sNUR (mgNO₃-N/gVSS·h)	sPUR (mgP/gVSS·h)	sPRR (mgP/gVSS·h)
NO_2-N	4.93 ± 1.29		3.85 ± 1.11	4.09 ± 0.82
NO_3-N		2.02 ± 1.13	3.19 ± 0.78	1.99 ± 0.56

4. Conclusions

The type and concentration of external organic carbon source critically affected the via nitrite phosphorus uptake and release rates. Specifically, the increase of sCOD supplied by the external carbon source in the mixed liquor increased the via nitrite phosphorus removal. The use of OFMSW FL as external carbon source resulted in the highest sPUR and sPRR since it contained a mixture of acetic acid, propionic acid and butyric acid in suitable proportions and at much higher concentrations compared to the VFW FL. The enrichment of VFW FL with propionic acid and butyric acid resulted in an increase of the sPUR and sPRR. Initial nitrite concentrations up to 50-70 mg/L did not adversely impact on the via nitrite sPUR. However, higher nitrite concentration in the range of 100-120 mgNO₂-N/L resulted in some sPUR inhibition. Significant higher nutrient removal rates were obtained in the presence of NO_2-N instead of NO_3-N as electron acceptor and VFW FL FL enriched with SCFAs was used as external carbon source in all cases in both cases.

Acknowledgments

Nomenclature

Symbol	Meaning
COD	Chemical oxygen demand
DNBPR	Denitrifying via nitrite biological phosphorus removal
DO	Dissolved oxygen
DPAOs	Denitrifying phosphorus accumulating organisms
EBPR	Enhanced biological phosphorus removal
FL	Fermentation liquid
HAc	Acetic acid
HBut	Butyric acid
HPr	Propionic acid
N	Nitrogen
NH_4-N	Ammonium
NO_2-N	Nitrite
NO_3-N	Nitrate
NO_x-N	Nitrate and nitrite
OFMSW	Organic fraction of municipal solid waste
P	Phosphorus
PAOs	Phosphorus accumulating organisms
PHAs	Polyhydroxyalkanoates
PO_4-P	Phosphate

SBR	Sequencing batch reactor
SCFAs	Short chain fatty acids
sCOD	Soluble COD
sNUR	Specific nitrite uptake rate
sPRR	Specific phosphorus release rate
sPUR	Specific phosphate uptake rate
TKN	Total Kjeldahl nitrogen
TSS	Total suspended solids
TVS	Total volatile solids
UASB	Upflow anaerobic sludge blanket
VFW	Vegetable and fruit waste
VSS	Volatile suspended solids

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