On-line titrimetric monitoring of anaerobic–anoxic EBPR processes

M. Vargas, A. Guisasola, J. Lafuente, C. Casas and J. A. Baeza

ABSTRACT

Denitrifying phosphorus accumulating organisms (DPAO) are able to remove nitrogen and phosphorus simultaneously. The use of DPAO in EBPR systems results in a substantial saving on aeration cost and a lower sludge production when compared to anaerobic–aerobic EBPR systems. This process is usually studied in sequencing batch reactors (SBR) and monitored with off-line measurements. However, off-line monitoring implies low frequency data sampling and delay between sampling and obtainment of the results. For this reason, an online measurement such as titrimetry is strongly recommended to improve the daily management of the lab-scale SBR. This paper shows different applications of titrimetric measurements for on-line monitoring of DPAO lab-scale SBR cycles. The results demonstrate that titrimetry is a suitable tool for detecting the end of phosphorus release and carbon substrate depletion point in the anaerobic phase. Moreover, this paper proposes the indirect measurement of nitrate/nitrite uptake rate with titrimetric measurements, which allows the on-line estimation of its concentration during the anoxic phase. Therefore, titrimetry is an on-line measurement with a high potential to implement new control strategies in DPAO lab-scale SBR systems.

Key words | DPAO, EBPR, monitoring, nitrate, nitrite, titrimetry

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INTRODUCTION

The enhanced biological phosphorus removal (EBPR) process is based on the enrichment of activated sludge with polyphosphate accumulating organisms (PAO). This process is usually conducted under sequential anaerobic and aerobic conditions so that a physical separation between the electron donor (organic matter) and the electron acceptor (oxygen) is obtained and thus, PAO are favoured against other microorganisms. However, it is widely known that a fraction of PAO, called denitrifying PAO (DPAO) is capable to use nitrate as electron acceptor if oxygen is not available (e.g. Hu *et al.* 2002; Zeng *et al.* 2003*a*, among many others).

DPAO are very interesting in terms of biological nutrient removal since nitrate reduction and phosphorus uptake can be achieved simultaneously (Murnleitner *et al.* 1997). The DPAO pathway is highly beneficial since this process implies reduced aeration cost as well as lower cell yield and sludge production than PAO (Zeng *et al.* 2003*b*). In addition, nitrite is also used as electron acceptor in the anoxic uptake process (Hu *et al.* 2003; Saito *et al.* 2004). DPAO can be enriched under sequential anaerobic and anoxic conditions using lab-scale SBR where the sludge is maintained in a properly controlled environment. The SBR cycle is usually monitored with off-line measurements such as volatile fatty acids (VFA), nitrate/nitrite, phosphate, and sometimes, internal storage polymers. However, off-line monitoring is not efficient enough owing to the low frequency of data sampling and the existing delay between sampling and obtainment of results (Guisasola *et al.* 2007). The on-line monitoring of a DPAO lab-scale SBR is not a straightforward issue, since several acid/base species are involved and respirometry is not available. Hence, there is a

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lack of a proper on-line measurement able to cope with these systems.

The anaerobic chemical and biochemical processes of DPAO metabolism involve acid or base species and thus, they have a strong influence on pH. Although pH can be used as an output variable for on-line monitoring (Serralta *et al.* 2004), the variation in the buffer compounds concentration (a very common scenario in biological systems) results in different pH absolute variations for the same process. This fact decreases the quality of the data obtained using pH as on-line measurement. On the other hand, titrimetry can be a suitable tool for on-line monitoring of the process because proton production can be calculated even with highly buffered media. This technique consists of the indirect measurement of the proton production (or consumption) through the monitoring of the amount of base (or acid) dosage necessary to maintain a constant pH. Titrimetric monitoring applications are found in the literature for organic matter degradation or ammonium oxidation, but only recently this technique is suggested for EBPR monitoring (Yuan *et al.* 2006; Guisasola *et al.* 2007).

This paper illustrates the theoretical link between DPAO metabolism and titrimetric measurements. In addition, several applications for on-line titrimetric monitoring of a DPAO SBR are presented. The results show that this technique is a reliable tool to monitor the anoxic nitrate or nitrite consumption rate. The titrimetric monitoring (i) simplifies the daily management of the reactors, (ii) enables the early detection of abnormal situations such as an equipment failure and (iii) allows a future implementation of control strategies for the process improvement.

METHODS

Equipments and operation

A fully monitored anaerobic–anoxic SBR (oxygen, pH, ORP and temperature) with a PLC (SIMATIC S7-226, Siemens) on top of the control system was used to enrich the sludge with DPAO. The 10 l reactor was inoculated with an active PAO sludge withdrawn from an anaerobic-aerobic SBR previously described in Guisasola *et al.* (2007). The DPAO SBR was operated during seven months with 3 cycles per day. Each cycle consisted of 120 min of anaerobic phase (5 initial min for feeding), 300 min of anoxic phase, 55 min of settling and 5 min to extract 5 l of the supernatant, producing a hydraulic residence time of 16 h. The sludge residence time was kept at 15 d by periodic wastage during the end of the anoxic period. A fixed nitrogen gas flow was sparged during both anaerobic and anoxic phases to avoid oxygen surface transfer. The temperature was controlled at 25 ± 1°C. HCl (1M) and NaOH (1M) were added to control the pH at 7.50 ± 0.05. Two separate solutions called "concentrated feed" (0.10 l) and "P-water" (4.75 l) collectively formed the synthetic wastewater used in this study. The "concentrated feed" used contained propionic acid as COD and its composition was the same as in Guisasola *et al.* (2007). The P-water consisted of (mg/l RO water): 54.4 KH_2PO_4 and 41.2 K_2HPO_4. Consequently, the synthetic wastewater had a concentration of 20 mg/l of PPO_4^{3-}. 0.15 l of N-solution ($NaNO_3$ or $NaNO_2$) was added at the beginning of the anoxic phase to achieve the required initial N-concentration in the reactor.

Titrimetric measurements

Titrimetry is the indirect measurement of the total proton production (HP) of a certain process. HP is obtained monitoring the acid and base dosage profiles required to maintain the pH constant (Equation 1)

$$HP = V_{BASE} \cdot C_{BASE} - V_{ACID} \cdot C_{ACID} \quad (1)$$

where V_{BASE} and V_{ACID} stand for the accumulated base and acid dosage (ml) and C_{BASE} and C_{ACID} for base and acid concentration (M). Titrimetric technique was implemented in the SBR using peristaltic dosage pumps (18.18 ml/min). The proton production rate (HPR) is also used as a monitoring tool in some studies dealing with titrimetric measurements. HPR is calculated as the first derivative of HP with respect to time.

Chemical analysis

Analyses of phosphate, nitrate and nitrite in filtered samples were performed with an Electrophoresis Capillar System

(Quanta 4000E CE – WATERS). The electrolyte used was a commercial solution (Ionselect High Mobility Anion Electrolite). Propionic acid was measured by Liquid Chromatography (HPLC). A Hewlett Packard 1050 equipped with a Bio Rad Aminex HPX-87H ion exclusion column at 25 °C was used. Mixed liquor suspended solids (TSS) and mixed liquor volatile suspended solids (VSS) were analysed according to standard procedures (APHA 1995).

RESULTS AND DISCUSSION

Titrimetry as a tool for DPAO monitoring

The total proton production (HP_{TOT}) of a certain process is the sum of the HP of the involved subprocesses calculated as in Equation 1. Guisasola *et al.* (2007) described the HP contribution of the main subprocesses involved in the PAO anaerobic-aerobic metabolism. The main difference between PAO and DPAO metabolism is the electron acceptor. In the PAO scenario, oxygen uptake does not have any effect on pH (i.e. HP = 0). On the other hand, DPAO metabolism involves nitrate and/or nitrite uptake, which results in a system basification, since both nitric and nitrous acids are strong. Table 1 shows the HP of each subprocess involved in the DPAO metabolism.

Theoretically, HP in the anaerobic phase should be positive (i.e. base should be dosed to control pH) according to Table 1 if the typical P/VFA and CO_2/VFA molar ratios were used. These ratios are 0.42 and 0.12, respectively for propionate as sole carbon source (Oehmen *et al.* 2005). However, most of these systems are sparged with nitrogen gas to obtain anaerobic conditions and CO_2 stripping occurs. Guisasola *et al.* (2007) showed that the system basification linked to CO_2 stripping may become the predominant effect on pH and then, the measured HP under anaerobic conditions becomes negative (see Figure 1).

During the anoxic phase, the system is highly basified as phosphoric, nitric and nitrous acids are taken up and hence, acid addition is supposed to be dosed to control the pH. Moreover, an additional system basification due to CO_2 stripping can be observed if nitrogen gas is used in this phase to avoid oxygen transfer.

Table 1 | Effect of DPAO metabolism subprocesses on the pH and associated proton production (HP) for pH values around 7.5. VFA_{UPTAKE}, $P_{RELEASE}$, $CO_{2\ PRODUCTION}$, $CO_{2\ STRIPPING}$, P_{UPTAKE}, $NO^-_{3\,REDUCTION}$, $NO^-_{2\,REDUCTION}$ and N_{UPTAKE} are in molar basis

Phase	Subprocess	Effect on pH	HP (mmol H^+)	Acid constants (298 K)
Anaerobic	VFA uptake	Basification	$-(VFA_{UPTAKE})/(1+10^{pK_{VFA}-pH})$	$pK_{VFA} = 4.9$ (propionic acid)
	CO_2 stripping	Basification	$-(CO_{2\,STRIPPING})/(1+10^{pK_{C1}-pH})$	$pK_{C1} = 6.36$
	CO_2 production	Acidification	$(CO_{2\,PRODUCTION})/(1+10^{pK_{C1}-pH})$	$pK_{C1} = 6.36$
	Phosphorus release	Acidification	$(P_{RELEASE})/(1+10^{pK_{P2}-pH})$	$pK_{P2} = 7.20$
Anoxic	Phosphorus uptake	Basification	$-(P_{UPTAKE})/(1+10^{pK_{P2}-pH})$	$pK_{P2} = 7.20$
	NO_3^- to NO_2^- reduction	Basification	$-(NO_{3\,REDUCTION})/(1+10^{pK_{NO_3}-pH})$	$pK_{NO_3} = 2.00$
	NO_2^- to N_2 reduction	Basification	$-(NO_{2\,REDUCTION})/(1+10^{pK_{NO_2}-pH})$	$pK_{NO_2} = 3.34$
	Ammonia uptake for growth	Acidification	$(N_{UPTAKE})/(1+10^{pK_{NH3}-pH})$	$pK_{NH_3} = 9.25$
	CO_2 stripping	Basification	$-(CO_{2\,STRIPPING})/(1+10^{pK_{C1}-pH})$	$pK_{C1} = 6.36$
	CO_2 production	Acidification	$(CO_{2\,PRODUCTION})/(1+10^{pK_{C1}-pH})$	$pK_{C1} = 6.36$

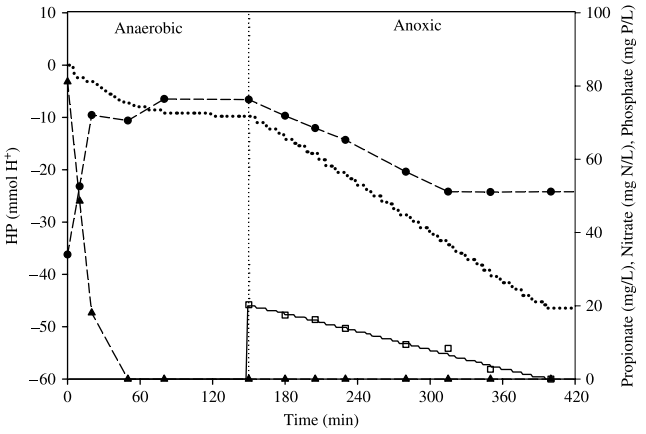

Figure 1 | Typical SBR anaerobic–anoxic cycle with nitrate as electron acceptor monitored with titrimetric measurements (dotted line). Off-line measurements: propionic acid (▲), nitrate (□) and phosphate (●). The estimation of nitrate concentration based on HP measurements is represented with a solid line.

On-line monitoring of DPAO metabolism with nitrate as electron acceptor

Figure 1 shows a typical anaerobic–anoxic cycle performed in the SBR with a DPAO enriched sludge, propionate as the sole carbon source and nitrate as the electron acceptor. Table 2 summarises the initial values and the experimental conditions for this experiment.

At the beginning of the anaerobic phase (Figure 1), the proton consumed due to propionate uptake and CO_2 stripping (Table 1) was higher than the proton produced due to phosphorus release and CO_2 production. As a result, acid dosage was required indicating a decrease in HP. After the propionate was depleted (around minute 50), acid was still dosed as CO_2 stripping was the most predominant effect on pH (from minute 50 to 80). When phosphorus was totally released, the net CO_2 production was negligible and HP remained almost unchanged until the anoxic phase

Table 2 | Initial values for the anaerobic–anoxic batch experiment with nitrate as electron acceptor

Initial values		Experimental Conditions	
Propionate	100 mg/l	VSS	1.3 g/l
P	10 mg P/l	TSS	1.64 g/l
Nitrate ($t = 150$ min)	20 mg N − NO_3^-/l	T	25 °C
		pH	7.5

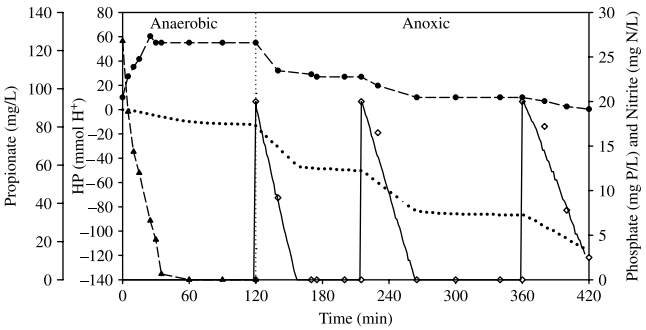

Figure 2 | Typical SBR anaerobic–anoxic cycle with nitrite as electron acceptor monitored with titrimetric measurements (dotted line). Off-line measurements: propionic acid (▲), nitrite (◇) and phosphate (●). The estimation of nitrite concentration based on HP measurements is represented with a solid line.

(at minute 150). Under anoxic conditions, a high acid dosage was needed as a consequence of phosphorus uptake, nitrate and nitrite reduction, and CO_2 stripping. Thus, HP value dropped significantly but stopped at the same time to nitrate depletion (around minute 400). Finally, the rest of subprocesses stopped from nitrate depletion on and thus, HP remained almost constant.

The experimental results showed that nitrate reduction was the dominant subprocess on the HP profile under anoxic conditions. Nitrate presence is the switching factor for the occurrence of some processes such as phosphorus uptake, glycogen production and biomass growth from PHA. Then, nitrate uptake rate (NO_3UR) could be predicted using HP measurements (see Figure 1). NO_3UR and HPR were constant in the range of 150 to 230 and they could be calculated using linear regressions, obtaining values of -0.078 mg N − NO_3^-/l minand 0.142 mmol H^+/min, respectively. Hence, the NO_3UR/HPR ratio was calculated as $K_{NO_3} - HP = 0.546$ mg N − NO_3^-/l mmol H^+. This ratio indicates the decrease in nitrate concentration per mmol of H^+ added in the monitored system. $K_{NO_3} - HP$ can

Table 3 | Initial values for the anaerobic–anoxic batch experiment with nitrite as electron acceptor

Initial values		Experimental conditions	
Propionate	125 mg/l	VSS	1.3 g/l
P	10 mg P/l	TSS	1.5 g/l
Nitrite ($t = 120$, 215 and 360 min)	20 mg N − NO_2^-/l	T	25 °C
		PH	7.5

Table 4 | Initial values for the anaerobic–anoxic batch experiment with nitrate and nitrite

Initial values (nitrate experiment)		Experimental conditions	
Propionate	100 mg/l	VSS	1.1 g/l
P	10 mg P/l	TSS	1.2 g/l
Nitrate ($t = 150$ min)	20 mg $N-NO_3^-$/l	T	25 °C
		PH	7.5
Propionate	80 mg/l	VSS	0.5 g/l
P	10 mg P/l	TSS	0.54 g/l
Nitrite ($t = 120$ min)	25 mg $N-NO_2^-$/l	T	25 °C
		PH	7.0

be considered constant when similar conditions such as pH, temperature or nitrogen gas sparging flow are maintained in the SBR. Therefore, nitrate concentration at any time was calculated (Equation 2) if the initial concentration was known.

$$c^t_{N-NO_3} = c^{t0}_{N-NO_3} + K_{NO_3-HP}\cdot(HP^{t0} - HP^t) \quad (2)$$

Figure 1 shows the estimation of nitrate concentration (solid line) based on HP measurements considering the initial nitrate concentration at the beginning of the anoxic phase. An excellent agreement between experimental and estimated nitrate concentration values was obtained. This on-line estimation would allow implementing new control strategies as for example 1) on-line length adaptation of the anoxic phase (finishing this phase when nitrate depletion is detected) or 2) online control of the nitrate concentration during this phase manipulating the amount of nitrate dosed.

On-line monitoring of DPAO metabolism with nitrite as electron acceptor

Figure 2 illustrates an anaerobic–anoxic cycle experiment with nitrite as electron acceptor and titrimetric measurements with a setpoint pH of 7.5. Table 3 shows the initial values and the experimental conditions.

In the anaerobic phase, the predominant events were VFA uptake and CO_2 stripping (Table 1). Consequently, HP was negative indicating that acid was added to control the pH at 7.5. After propionic acid depletion, a much lower acid addition was still necessary because of CO_2 stripping. The SBR was switched to anoxic conditions when nitrite was added at $t = 120$. HP value dropped till the first nitrite depletion point (around minute 170). Phosphorus concentration decreased simultaneously with nitrite concentration, remaining constant after nitrite depletion. HP remained constant when nitrite was not present, indicating that the effect of the rest of the subprocesses was negligible. When the second and third nitrite pulses were added (at 215 and 360 minutes, respectively), the profiles of the on-line and off-line measurements followed the same pattern described above.

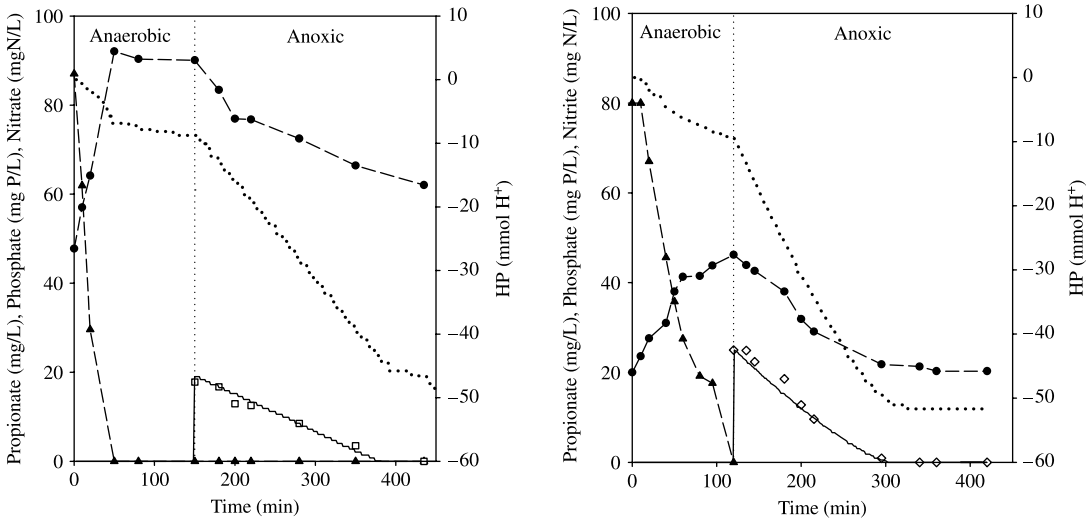

Figure 3 | Typical SBR anaerobic–anoxic cycles monitored with titrimetric measurements (dotted line). Off-line measurements: propionic acid (▲), nitrate (□), nitrite (◇) and phosphate (●). The estimations of nitrate and nitrite concentration based on HP measurements are shown with a solid line.

Nitrite uptake rate (NO_2UR) and HPR were estimated with experimental data between 120 and 140 minutes as described in the previous section (0.54 mg $N-NO_2^-$/l min^{-1} and 0.882 mmol H^+/min, respectively). A constant ratio $K_{NO_2}-HP = 0.611$ mg $N-NO_2^-$/l mmol H^+ was obtained. Nitrite concentration in time was estimated knowing the amount of nitrite added in each pulse (Equation 2), as shown in Figure 2 (solid line). Again, the estimated concentrations fitted fairly accurately to the experimental values.

Experimental validation of the on-line concentration estimation based on HP

Different SBR cycle experiments were carried out in order to validate the proposed methodology. Table 4 shows the initial values of two experiments with nitrate or nitrite as electron acceptor, while Figure 3 depicts the experimental profiles obtained.

Titrimetric measurements in both anaerobic phases (Figure 3) were strongly related with the end of phosphorus release and the VFA depletion point. HP value was also clearly linked to the nitrate or nitrite consumption. The solid lines represent the predicted nitrate or nitrite concentration using the initial concentration, HP and the $K_{NO_3}-HP$ or $K_{NO_2}-HP$ ratios calculated above from different experiments. It was observed that both nitrate and nitrite profiles were accurately predicted.

CONCLUSIONS

Titrimetry is a reliable tool to monitor the anaerobic VFA uptake and also the anoxic nitrate or nitrite consumption linked to phosphorus uptake, glycogen production and biomass growth from PHA. Titrimetric monitoring is easily implemented if a pH control is already available, only supervising the amount of acid and base added. Nitrate or nitrite consumption rate are estimated in a SBR with a calibration of the Nuptake rate/HPR ratio using previous experiments. These ratios can be assumed constant when similar conditions such as pH, temperature or nitrogen gas sparging flow are maintained in the SBR. As a result, if the initial nitrate or nitrite concentration is known, the on-line titrimetric estimation of its concentration during the cycle is obtained. Therefore, this information can be used to implement new process control strategies, as for example 1) on-line cycle-length adaptation (modifying the anaerobic or anoxic phase duration) or 2) on-line control of the nitrite or nitrate concentration during the anoxic phase.

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