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Biological Phosphorus Removal and Denitrification of a Fish Farm Effluent in a Sequencing Moving Bed Biofilm Reactor

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Removal of phosphate and nitrate from the effluent of a fish farm with a recirculation system was tested in a sequencing moving bed biofilm bioreactor (SMBBR) over a 160-day period. This bioprocess made use of a stock tank (ST) that allowed the same volume of anaerobic water to be reused from one batch to another. Water from the ST contained an excess of a carbon source (acetate), which made it possible to alternate between anaerobic (1.5 h) and aerobic/anoxic (4 h) conditions to achieve enhanced biological phosphorus removal (EBPR). The developed biomass (2,072 mg total solids $m^{-2}{\cdot}d^{-1}$ and 892 mg total volatile solids $m^{-2}{\cdot}d^{-1}$) removed 7.5 mg of PO_4-P per litre and 8.5 mg of NO_3-N per litre from an influent containing 10 mg of PO_4-P per litre and 21 mg NO_3-N per litre. The dynamic variation of phosphate and chemical oxygen demand (COD) in the influent showed that the biomass was sensitive to the $COD_{available}/P_{influent}$ ratio. A ratio of 10 to 15 mg of COD per milligram of P seemed to favour phosphorus accumulating organisms (PAOs). Differences between the nitrate, phosphate, and oxygen reduction kinetics suggested that the denitrification could be attributed to organisms other than PAOs. The SMBBR-ST showed potential for EBPR and for denitrification as well. However, the economic feasibility of implementing such a process in a full-scale operation remains to be demonstrated.

Key words: **wastewater, biological phosphorus removal, denitrification, biofilm, moving bed, fish farming**

Introduction

Phosphorus (P) and nitrogen (N) are the two main pollutants of fish farm effluents. Fish farms operated as a partially closed circuit (PCC) or closed circuit (CC) require a nitrification process because ammonia (NH_3) produced by the fish is toxic to them. PCC effluents contain up to 25 mg of NO_3-N per litre. Water in CC systems may reach concentrations greater than 50 mg of NO_3-N per litre and 20 mg of PO_4-P per litre (Trépanier et al. 2002).

Chemical precipitation is the most common process for removing phosphate from wastewater. However, that process cannot be used in fish farms run in PCC or CC. An alternative to chemical phosphorus removal is enhanced biological phosphorus removal (EBPR) whereby phosphorus accumulating organisms (PAOs) accumulate phosphorus beyond their metabolic requirements. For EBPR to occur, the biomass must be exposed to alternating conditions: anaerobic conditions in the presence of volatile fatty acids (VFAs) followed by aerobic/anoxic conditions (Comeau et al. 1986; Smolders et al. 1995; Kuba et al. 1996; Mino et al. 1998; Oehmen et al. 2007).

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EBPR in activated sludge processes is a well proven technology and is used reliably to treat municipal wastewater. EBPR in attached growth processes (biofilm) is an alternative to activated sludge processes and has shown promising potential in the recent times (Gonzalez-Martinez and Wilderer 1991; Goncalves and Rogalla 1992; Rovatti et al. 1995; Garzon-Zuniga and Gonzalez-Martinez 1996; Rogalla et al. 2006). However, EBPR by biofilms has only been tested at the laboratory or pilot scale due to the complexity of exposing the biomass to alternating anaerobic and aerobic/anoxic conditions.

Sequencing moving bed biofilm reactors (SMBBRs) allow for flexible operations and are convenient when a number of biological processes are involved, as was the case in the present study. The presence of nitrate in the influent remains a constraint for EBPR since it favours the development of ordinary denitrifying organisms at the expense of PAOs. To cope with the presence of nitrate in fish farm effluents, a stock tank (ST) was combined with a SMBBR in this study. The ST contained an aqueous solution of sodium acetate maintained under anaerobic conditions. This type of SMBBR has been shown to be efficient at the pilot scale for the denitrification of a CC system (Labelle et al. 2005; Dupla et al. 2006).

Vallet (2007) tested the SMBBR process with a real saltwater fish farm effluent from the marine St. Lawrence mesocosm at the Montreal Biodome, but was unsuccessful, possibly due to high calcium content

of the water. He also tested the process on a synthetic freshwater effluent, but phosphate removal efficiency was low (20%) and denitrification was minimal.

The goal of the present study was to test the SMBBR-ST process in real fish farming conditions using the effluent from a rainbow trout breeding unit. An SMBBR-ST unit was installed in the Laboratoire Régional des Sciences Aquatiques (LARSA) at Université Laval in Quebec City. The first objective was to acclimate the attached biomass developed by Vallet (2007) at the Montreal Biodome to fish farming conditions, and to show the feasibility of using PAOs and denitrifying PAOs to remove nitrate and phosphate from a fish farm effluent. The second objective was to optimize the operating conditions of the SMBBR, mainly the acetate concentration to be maintained in the ST, and the duration of the operating cycle phases.

Material and Methods

Process Configuration

A 110-L moving bed bioreactor adapted from Labelle et al. (2005) was used for this study (Fig. 1). It was paired to a 220-L ST to store the anaerobic concentrate. The reactor was filled with 800 polypropylene carriers (Cascades-1A, Koch-Glitsch, Wichita, Kans., U.S.A.). Each carrier had a specific surface area of 19 cm^2 and a density of 0.95 $kg \cdot dm^{-3}$. The carriers were chosen following a fluidization study done by Dupla et al. (2006) in the reactor used for the present project. The carriers were retained at the surface of the reactor by a 45-degree-angle conical screen. A 9.5-mm eductor (model TME, Penberthy Inc., Prophetstown, Ill., U.S.A.) positioned above the screen propelled the carriers to the bottom of the reactor, thereby mixing the entire reactor. The volume under the screen (working volume) was 65% of the total reactor volume. Sampled carriers used for biomass quantification and for batch tests were replaced by new carriers to maintain 800 plastic carriers in the bioreactor.

Filling of the reactor was controlled by a 12.7-mm solenoid valve (Asco Red Hat, 8210 series, Brantford, Ont., Canada). The anaerobic concentrate was recirculated and transferred from the ST to the reactor via centrifugal magnetic drive pumps (models TE-5-MD-SC, Little Giant Pump Co., Oklahoma City, Okla., U.S.A. and AC-4C-MD, March MFG Inc., Glenview, Ill., U.S.A., respectively). The concentrate was returned to the ST by gravity. An automated three-way transfer valve (QAT-CENTREOFF, Chemline Plastic Limited, Thornhill, Ont., Canada) allowed the reactor to be filled and emptied. A pH probe (DynaProbII pH, Broadley-James Corp., Irvine, Calif., U.S.A.) and a dissolved oxygen probe (Oxymax W COS61, Endress + Hauser, Burlington, Ont., Canada) were installed on the recirculation line. Process control and data acquisition were carried out using LabVIEW (version 7.1, National Instruments Corporation, Austin, Tex., U.S.A.) and an acquisition module OMB-DAQ-56 (OMEGA Engineering, Inc. Stamford, Colo., U.S.A.).

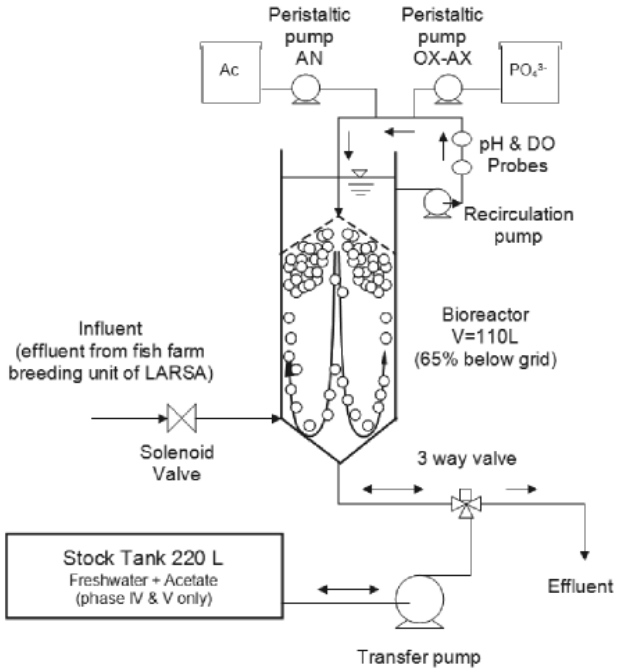

Fig. 1. Process diagram for SMBBR with ST.

Typical Operating Cycle

The SMBBR process was run in 308 to 368 min/cycles, thus permitting 4.7 to 3.9 cycles per day. Each cycle involved an aerobic/anoxic phase and an anaerobic phase. The reactor was emptied between the phases but the biomass and intracellular compounds remained on the plastic carriers. This process configuration and operating mode theoretically eliminated the presence of nitrate—found at a high concentration in the influent—during the anaerobic phase. Six periods were involved in one cycle: 1) filling period (18 min), 2) aerobic/anoxic period (3 to 4 h duration, depending on the operating conditions); 3) emptying period (8 min); 4) pump transfer period (3 min); 5) anaerobic period (90 min); and 6) gravity transfer period (9 min).

During the filling period, the effluent from the PCC fish farming unit located at LARSA was pumped (40 L/h) into a 300 L well-mixed Plexiglas tank from which the reactor was fed by gravity via the solenoid valve. During the aerobic/anoxic period, the electron acceptors, oxygen and nitrate, were consumed and phosphate was accumulated in the biomass as polyphosphates (poly-P) using intracellular polyhydroxyalkanoates (PHAs) stored during the anaerobic phase. During the emptying period, the treated effluent flowed to the drain by gravity via the three-way valve. During the pump transfer period, the concentrate containing excess acetate was pumped to the reactor. During the anaerobic period, acetate was stored as PHAs by bacteria using poly-P and glycogen, and phosphate was released in the water. During the gravity transfer period, the concentrate in the reactor was emptied to the ST by gravity via the three-way valve. So doing, the phosphate concentration in the ST increased after each cycle.

TABLE 1. Wastewater characteristics of a PCC fish farm breeding unit at LARSA

Parameter	*Units*	*Value*	*Standard deviation*	*n*
COD_{total}	mg/L	33.5	11.9	4
$COD_{filtered}$	mg/L	26.8	6.3	4
TSS	mg/L	0.9	0.5	2
$TKN_{filtered}$	mg N/L	1.2	0.6	5
NH_4^+	mg N/L	0.09	0.1	5
NO_3^-	mg N/L	24.7	3.7	17
NO_2^-	mg N/L	<0.005	0	13
P_{total}	mg P/L	1.9	0.6	5
PO_4	mg P/L	1.6	0.6	17
Na^+	mg/L	328	0	1
K^+	mg/L	5.0	1.0	5
Ca^{2+}	mg/L	16.5	3.1	5
Mg^{2+}	mg/L	2.7–7.7 [a]	0.4	5
SO_4^{2-}	mg/L	26.8	2.7	2
Cl^-	mg/L	290	15.0	2
Fe	mg/L	<0.05	0	2
Alkalinity	mg $CaCO_3$/L	88.1	29.1	5
Hardness	mg $CaCO_3$/L	60.2	9.9	5
Salinity	—	<2.0	0.2	5
Conductivity	µS	1,290–3,180		
pH	—	7.7	0.2	17
O_2	mg/L	10.8	0.2	17
Temperature	°C	14	0.2	17

[a]Magnesium ($MgCl_2$) was added during the last two weeks of pilot operation (run V).

Wastewater Composition

The fish farming wastewater treated in this study originated from a LARSA rainbow trout breeding unit with a 30 kg/m^3 fish load. The volume of water in the breeding unit was 9 m^3. The water was renewed at a 40 L/h rate, thus making the unit a PCC. A water treatment system comprised of sand filters and a nitrifying biofilter ensured water quality in the breeding unit. The pH was adjusted to 7.5. The effluent typically contained 24.7 mg of NO_3-N per litre, 1.6 mg of PO_4-P per litre, and 26.8 mg/L of filtered chemical oxygen demand (COD) (Table 1).

Experimentation

Since the biomass developed by Vallet (2007) in his freshwater essay showed significant—although low—P removal activity, it was transferred to the SMBBR to speed up the start up. The biomass was acclimated for 20 days to the fish farming conditions by alternating the anaerobic (with acetate) and aerobic/anoxic phases. An experimental plan composed of five runs was then conducted over the course of 160 days (Table 2).

Two different protocols were used to supply acetate to the SMBBR. During runs I to III, a sodium acetate solution of 100 g of COD per litre was injected into the reactor during the first 2 min of the anaerobic phase using a dual channel peristaltic pump (model C/L 77120-52, Cole-Parmer Masterflex, Vernon Hills, Ill., U.S.A.) to obtain an initial concentration of 70 mg of COD per litre. The fraction of acetate unconsumed during the

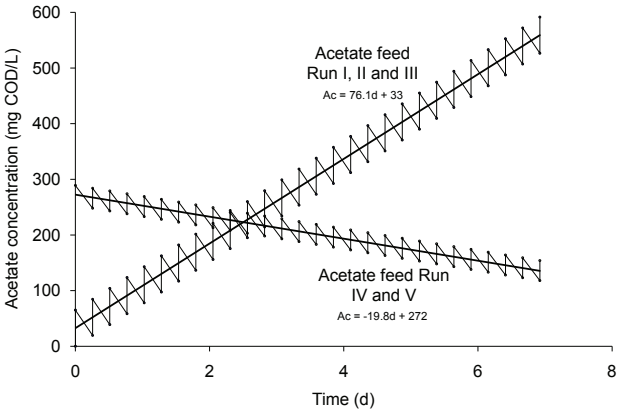

Fig. 2. Simulated changes in the ST's acetate concentration over a one-week operating sequence. The profile for each was calculated using an acetate uptake rate of 3 and 40 mg of COD per litre per cycle in the ST and reactor, respectively. Each cycle lasted 5.1 h.

TABLE 2. Description of the conditions during the five runs[a]

Run:	I Low P	II Med P	III High P	IV Ac build up	V Ox-Ax duration
Duration (days)	0–40	41–66	67–94	95–144	145–159
PO_4 influent (mg P/L)	1.5 ± 0.9	5.5 ± 1.0 [c]	10 ± 1.0 [c]	10 ± 1.0 [c]	10 ± 1.0 [c]
Acetate feed (see Fig. 2) [b]					
New ST water (mg COD/L)	70	70	70	300	300
Old ST water (mg COD/L)	600	600	600	100	100
Anaerobic phase (min)	90	90	90	90	90
Aerobic/anoxic phase (min)	180	180	180	180	240

[a] Three phosphorus levels were tested (low P in run I, medium P in run II and high P in runs III, IV and V). Along with the high P levels, acetate build-up in the ST was tested in run IV, whereas the impact of an increase in the duration of the aerobic/anoxic phase was tested in run V.
[b] Acetate feed - Old ST water corresponds to the COD in the ST at the end of a typical 7-day sequence (Fig. 2).
[c] Addition of P from a K_2HPO_4 solution.

anaerobic phase was accumulated in the ST as shown by the acetate concentration simulation in Fig. 2. During runs IV and V, acetate was injected directly into the ST (a 300-mg COD/L solution was prepared and renewed every week) and was also pumped into the reactor (pump injection) during the first 2 min of the anaerobic phase. The amount pumped was based on consumption during the typical anaerobic phase (approximately 40 mg/L). The phosphorus level was increased during runs II to V by pumping doses of potassium phosphate directly to the fish farm effluent.

Analytical Methods

Daily measurements of phosphate and nitrate were done using Phosver 3 and Nitraver 5 (HACH, Loveland, Colo., U.S.A.). Carbon measurements were done according to the HACH method (low range COD vials, <150 mg of COD/L). Cycles were monitored by sampling water in the reactor every 10 to 15 min. Samples were analyzed for phosphate, nitrate, nitrite, calcium, magnesium, and potassium by ion-exchange chromatography (Dionex Corporation, CS16 and AS19 analytical columns). All samples were filtered on 0.45-µm HA type Millipore filters. Total Kjeldahl nitrogen, ammonia, and total phosphorus were measured according to Standard Methods 4500-Norg D, 4500-NH3 H, and 4500-P G, respectively (APHA et al. 1998). Both total phosphorus and total Kjeldahl nitrogen were digested using the same digestion procedure (HgO catalyst).

Biomass Quantification

Reactor biomass was quantified through triplicate analysis of two carriers. Removed carriers were replaced by new ones to maintain a constant growth surface for the biofilm in the reactor. The biofilm and inorganic precipitates attached to the carriers were removed by sonication at 100% power (750 W) for 10 to 15 min in distilled water. Samples were dried at 105°C for 24 h to measure total solids (TS), followed by 2 h of combustion at 550°C to determine total inorganic solids. Total volatile solids (TVS) were measured by subtracting TIS from TS. Rather than total suspended solids (TSS), TVS were measured because sonication led to the partial solubilization of the TSS.

Mass Balances

Mass balances (percent mass out/mass in) for phosphorus and carbon were done over a seven-day sequence during run V (3.9 cycles/d). Samples were taken daily during that period. The nitrogen mass balance could not be calculated because the setup did not allow for the measurement of the gaseous nitrogen resulting from the denitrification process.

Results

Acetate consumption varied during the experimental plan (Fig. 3). Following biomass acclimation, it was less than 10 mg of COD per litre per phase, but from day 30 onwards, it varied between 20 and 50 mg of COD per litre per phase.

Phosphorus and NO_x (NO_2 + NO_3) concentrations were measured at the beginning and end of various cycles during the experimental plan (Fig. 4). When the phosphate concentration in the fish farm effluent was increased from 2 mg of P per litre to 5 mg of P per litre and 10 mg of P per litre, during runs II and III respectively, phosphate removal also increased. However, no significant NO_x removal was observed during runs I to III, meaning that no denitrification took place during these runs. Increasing the aerobic/anoxic phase duration from 3 to 4 h during run V did not improve system performance (Fig. 4).

From run IV onwards, acetate concentration in the ST was 300 mg of COD per litre, with a minimum of 100 mg of COD per litre (Fig. 2). Under those operating conditions, phosphate and nitrate removal reached 7.5 mg of P per litre and 8.5 mg of N per litre, i.e., 75 and 40% respectively (Fig.5). There seemed to be a

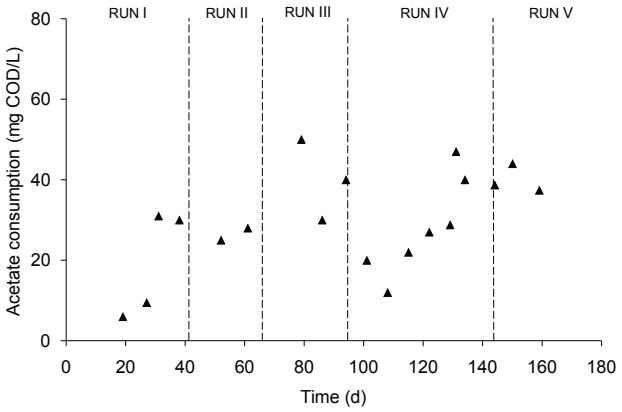

Fig. 3. Acetate consumption during the five experimental runs (anaerobic phase).

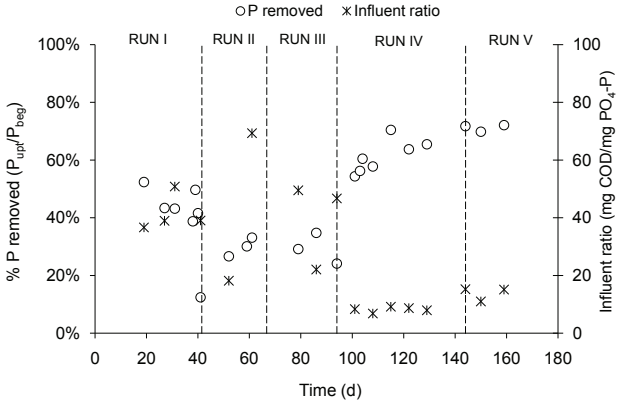

Fig. 5. Phosphate removal and $COD_{available}/PO4\text{-}P_{influent}$ ratios during the experiment's five runs.

correlation between the development of phosphorus removing biomass and the $COD_{available}/P_{influent}$ ratio. The best phosphorus removal took place at ratios of less than 20 mg of COD per mg of P (Fig. 5).

Phosphorus, nitrogen, and oxygen levels varied greatly over the course of a typical reactor cycle (Fig. 6). During the anaerobic phase, phosphate was released at a constant rate for 80 min, and then rapidly reached a plateau. Reduction in nitrate was linear throughout the aerobic/anoxic phase. Denitrification occurred in the presence of oxygen, and also when there was no more phosphorus removal. Very little nitrite was produced. The rate of oxygen consumption was constant during the first 90 min of the aerobic/anoxic phase, and it then decreased. Phosphate assimilation seemed to follow the same kinetics as oxygen.

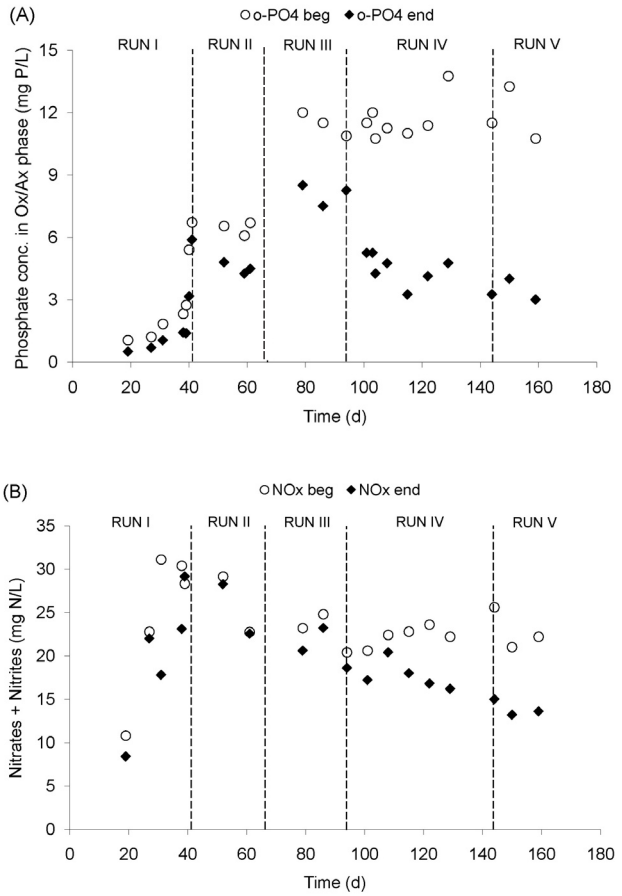

Fig. 4. Concentration of phosphates (A) and nitrates (B) at the beginning and end of the aerobic/anoxic phase.

Mass Balances

The accumulation rates for TS and TVS were estimated to be 2,072 mg of TS $m^{-2}\cdot d^{-1}$ and 892 mg of TVS $m^{-2}\cdot d^{-1}$, respectively, taking into account the active surface of 19 cm^2 per carrier where the biomass developed. Total phosphorus of the aerobic/anoxic phase effluent (330 mg of P), total phosphorus of the concentrate (21,340 mg of P), the biomass attached to the carriers (71.3 mg of P per gram of TS), and the accumulated biomass on the reactor wall (580 mg of P) and in the system piping (88 mg of P)

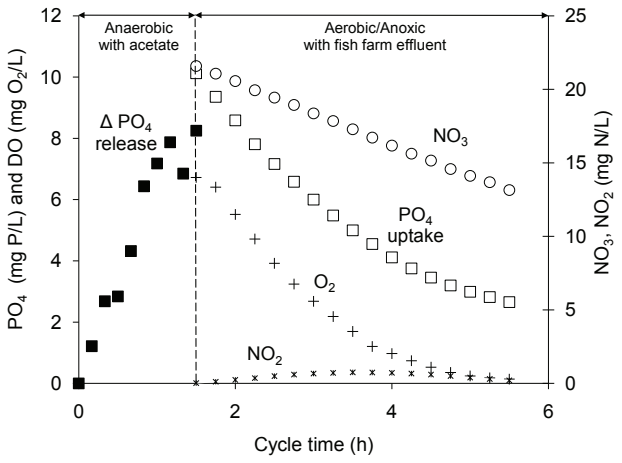

Fig. 6. Phosphate, oxygen, and ammonia levels in the reactor during an operating cycle. Results are from day 159 (run V) when the system was considered to be in a pseudostable state.

were measured. The resulting phosphorus mass balance (P out/P in) closed satisfactorily at 105% (Fig. 7).

The amount of phosphorus removed by biological means was estimated using the TS and TVS accumulation rates, taking into account the cellular growth requirements (0.02 g of P per gram of TVS) and the phosphorus content of the dry biomass (71.3 mg of P per gram of TS). This calculation showed that 91% of the phosphorus was removed biologically and that the remaining 9% was removed by other means such as chemical precipitation.

The only source of nitrogen present in the influent was nitrate. The presence of ammonia, nitrite, and organic nitrogen were considered negligible. As previously mentioned, up to 40% (8.5 mg of N per L) of nitrate was removed during run V through denitrification and cell growth. That removal occurred only during the aerobic/anoxic phase since the influent was never in contact with the ST concentrate.

The amount of nitrate removal was compared with the alkalinity produced. Based on a biomass nitrogen content of 6 to 12% (Rittmann and McCarty 2001), 0.5 mg of N per litre per phase was used for cell growth. The rest of it (8.0 mg of N per litre per cycle) was probably removed by denitrification since the measured amount of alkalinity produced during a cycle (28 mg as $CaCO_3$) was similar to the alkalinity known to be produced by denitrification (3.57 mg of $CaCO_3$ per mg of NO_3-N [Metcalf et al. 2003]).

The system's two carbon sources were acetate supplied during the anaerobic phase and carbon present in the fish farm effluent, which had a concentration of 26.8 mg of $COD_{filtered}$ per litre (Table 1). The carbon in the fish farm effluent was present only during the aerobic/anoxic phase. The average consumption determined during the aerobic/anoxic phase was 2 mg of COD per litre. Acetate was added by a metering pump (43.2 mg of COD per litre per phase) and also came from the acetate solution in the ST, which contained 289 mg of COD per litre. At the end of the seventh day, a residual concentration of 210 mg of COD per litre was measured in the ST. Average consumption of 37.4 mg of COD per litre occurred in the reactor during the anaerobic phase. In addition, consumption of 3.3 mg of COD per litre per phase was observed in the ST during the reactor's aerobic phase, which indicated that part of the detached biomass from the reactor was transferred to the ST. The COD mass balance closed satisfactorily at 99% (Fig. 7).

TABLE 3. Molar ratios (mol/mol) of cations cotransported with phosphate

	Direction of phosphate transport	
	Uptake	*Release*
Mg^{2+}/P	0.20	0.30
K^+/P	0.35	0.42
Sum of charges/P	0.75 [a]	1.02 [a]

[a] = 2 Mg^{2+}/P + K^+/P

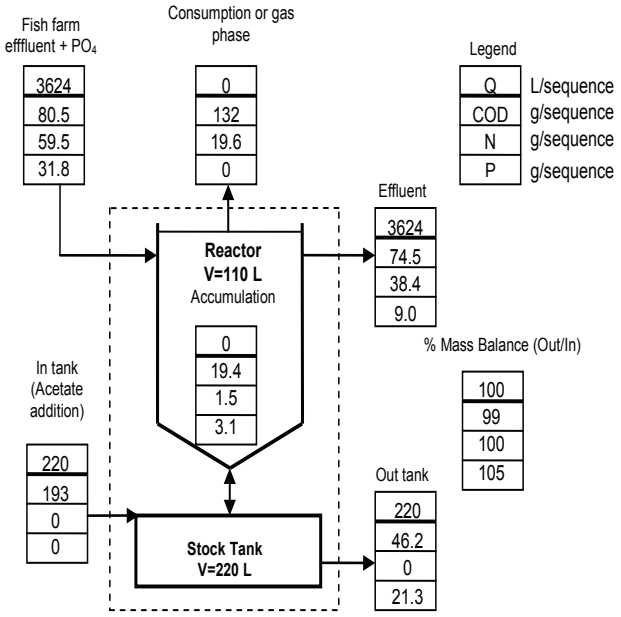

Fig. 7. COD, N, and P mass balance for a seven-day sequence, 5.1 h per cycle for run V.

Cations

Samples taken every 10 to 15 min during a typical cycle were analyzed for magnesium, potassium, and calcium (Table 3). Results showed that cations were released during the anaerobic phase and were taken up during the aerobic/anoxic phase. Thus, magnesium and potassium were cotransported with phosphates across the cell membrane, as observed by Comeau et al. (1987). Moreover, no release of calcium was detected. Although calcium seems to have an effect on biological phosphorus removal (Schonborn et al. 2001; Barat et al. 2006), several authors consider that it is not cotransported with phosphate (Pattarkine and Randall 1999; Schonborn et al. 2001; Rickard and McClintock 1992; Oehmen et al. 2007).

Discussion

Enhanced Biological Phosphorus Removal Efficiency

The SMBBR process achieved 75 and 40% removal efficiency for phosphate and nitrate, respectively. The biomass developed with this process is therefore able to remove phosphate present in the influent before releasing it to the ST. It can also consume acetate when the reactor is filled with ST water (anaerobic acetate solution). Magnesium and potassium cations also followed a pattern of uptake-release. All these are indications that EBPR occurred in the reactor.

One question remains, however: What proportion of phosphate removal can be attributed to actual biological

TABLE 4. Stoichiometric ratios of biological activity for each run

Run	$PO_{4,inf}$ *(mg P/L)*	$PO_{4,upt}$ *(mg P/L)*	$PO_{4,rel}$ *(mg P/L)*	COD_{upt} *(Mg/L)*	P_{rel}/COD_{upt} *(mol-P/mol-C)*	COD_{inf}/P_{inf} *(g/g)*	P_{rel}/TVS *(mg/g)*
I	1.8	0.8	1.6	31	0.05	51	1.1
II	6.3	1.9	1.1	22	0.05	74	0.9
III	10.8	2.6	2.0	40	0.05	47	1.6
IV	11.0	6.2	6.8	17	0.41	8	3.4
V	9.3	7.0	7.2	38	0.20	32	2.6

activity? The question is relevant because up to 80% of phosphorus removal has been attributed to precipitation in certain EBPR systems (Maurer and Boller 1999). An analysis of the biomass accumulation rate and phosphorus content showed that 91% of phosphate removal resulted from biological activity, and 9% from other phenomena such as phosphorus precipitation. On the other hand, if the analysis is based on cations that cotransport phosphates, i.e., if it is assumed that for every mole of phosphate removed, one mole of cations is taken up (Comeau et al. 1987), then 75% of the phosphate removal can be attributed to biological activity (Table 3).

Several elements can form complexes with phosphates, notably iron, aluminum, ammonium, and calcium (Snoeyink and Jenkins 1980). The first three elements could not have caused precipitation as their influent concentrations were low. Calcium could have been responsible for the precipitation that occurred in this study since a slight decrease of 1.1 mg of Ca per litre was observed during the aerobic/anoxic phase. The pH, 7.7 at the beginning and 8.2 at the end of the phase, was favourable to this precipitation reaction (Maurer and Boller 1999).

Denitrification

An organic carbon source is required for denitrification. The two organic carbon sources available in this experiment were dissolved organic carbon from the influent (27 mg of $COD_{filtered}$ per litre) during the aerobic/anoxic phase and acetate supplied during the anaerobic phase. The consumption of the first carbon source was less than 5 mg of COD per litre. The second carbon source was not directly available for the nitrate as it was only present during the anaerobic phase. However, if acetate was synthesized by organisms in the form of PHA, it would then be available for denitrification during the aerobic/anoxic phase because the biofilm remains in the reactor.

The contact between nitrate and the acetate is considered to be one of the main constraints of EBPR due to the competition between ordinary heterotrophic organisms and PAOs for VFAs uptake (Barker and Dold 1996; You et al. 2001; Bux et al. 2003). The ST was shown to be efficient in preventing contact between the biomass and substrate. The process therefore favoured the selection of organisms that synthesize acetate as PHAs, which could then serve as electron donors for organisms using either oxygen or nitrate as electron acceptors, thus making denitrification possible.

As previously mentioned, up to 40% (8.5 mg of N per litre) of the influent nitrates were removed, suggesting that denitrification was achieved using an intracellular carbon source such as PHA. Are these PAOs capable of using both oxygen and nitrate as electron acceptors? The slope of the nitrate curve in Fig. 6 shows that nitrate removal was constant, which indicates that anoxic conditions occurred within the biofilm when oxygen was present until the end of that phase. This implies that PAOs as well as other organisms capable of using nitrate as an electron acceptor were present in the biofilm.

The next question is whether or not those other organisms were denitrifying PAOs. The phosphate and oxygen curves (Fig. 6) suggest a decrease in their consumption kinetics, contrarily to the nitrate curve whose slope remained constant. Denitrification therefore was not achieved solely by PAOs. Other organisms could have carried out denitrification. Indeed, Zeng et al. (2003) suggested that glycogen accumulating organisms (GAOs) were able to denitrify, which could explain the excess acetate consumption observed in our experiment and which we will discuss later.

Consequently, the tested process has the potential to remove nitrate. However, the fact that phosphate uptake did not correspond to nitrate assimilation is cause for concern. Further studies on various carbon sources which could favour poly-P rather than glycogen synthesis are needed. For example, propionate requires less glycogen for PHA synthesis than acetate (Filipe et al. 2001; Zeng et al. 2002; Oehmen et al. 2007). If less glycogen is available, the organisms that use poly-P as an energy source for PHA synthesis would be favoured, and denitrifying or nondenitrifying PAOs would have a competitive advantage over GAOs.

Limitation of Low Phosphate Concentration and Effect of Acetate Concentration

The availability of phosphates in the influent is essential to guarantee sufficient synthesis of poly-P and thus sufficient energy for PHA synthesis under anaerobic conditions. The water from LARSA used in this study had a phosphate concentration of 2 mg of P per litre.

The SMBBR-ST did not have the capacity to increase this concentration as a sludge recirculation process would. To test whether the phosphate concentration was a limiting factor, the influent concentration was artificially increased to 5 mg of P per litre (run II) and to 10 mg of P per litre (runs III to V). System performance did not improve under those new operating conditions (Fig. 5). Therefore phosphate was not the limiting factor in this experiment.

As for the substrate, the supplied acetate concentration varied from 70 mg of COD per litre to 600 mg of COD per litre during the first three runs (Fig. 2). This was due to the accumulation in the ST of the acetate that was not entirely consumed during the anaerobic phase. This accumulation resulted in high $COD_{available}/P_{influent}$ ratios during runs I, II, and III. Several authors (Mino et al. 1998; Schuler and Jenkins 2003) have linked the influent COD/P ratio to the performance of EBPR systems. They suggested that an excess of COD (>50 mg of COD per mg of P) favours GAOs over PAOs, which become phosphate limited, whereas a ratio between 10 to 20 mg of COD per mg of P favours PAOs. Based on this hypothesis, the addition of acetate was adjusted and the acetate concentration was maintained between 100 and 300 mg of COD per litre during runs IV and V. As was the case for Mino et al. (1998) and Schuler and Jenkins (2003), the adjustment resulted in a rapid increase in the percentage of P removed (Fig. 5). Thus, not only was influent phosphate concentration important to the process, so was acetate concentration.

It should be noted that the biomass consumed acetate during the five runs, but the results were only conclusive during runs IV and V. During runs I, II, and III, the ratio of released phosphate to consumed acetate was minimal (0.05 mol of P per mol of C). In comparison, that ratio reached 0.21 and 0.41 mol of P per mol of C during runs IV and V, respectively (Table 4). This suggests that acetate was consumed by other organisms, possibly GAOs, which do not use poly-P hydrolysis as an energy source. Oehmen et al. (2007) studied various $P_{released}/COD_{assimilated}$ ratios and demonstrated that values less than 0.3 mol of P per mol of C were related to a high GAO percentage—either C*ompetibacter* or *Alphaproteobacteria*—whereas values over 0.3 mol of P per mol of C were related to a predominance of PAOs such as *Accumulibacter*. In fact, preliminary results from a 16S rRNA library showed the presence of sequences related to PAOs and GAOs (Bigras et al. 2008).

Given the clues indicating the presence of GAOs in the developed biomass, changing the carbon source could be considered a way to improve system performance and to reduce carbon consumption. Certain metabolic models (Filipe et al. 2001; Zeng et al. 2002; Oehmen et al. 2005) suggest that a smaller amount of glycogen is required to assimilate propionate than to assimilate acetate, which would favour the development of GAOs. It would therefore be interesting to test propionate as a carbon source or, better still, to alternate between the two carbon sources as suggested by Oehmen et al. (2007).

The process tested in this study showed potential for removing 75% of phosphate (7.5 mg of P per litre) and 40% of nitrate (8.5 mg of N per litre) from a fish farm effluent. Between 75 and 91% of phosphate removal resulted from biological activity, with the remaining fraction attributed to chemical precipitation. Cotransport of magnesium and potassium confirmed that there was biological activity.

The concentration of acetate supplied during the anaerobic phase greatly affected system performance; concentrations between 100 and 300 mg of COD per litre yielded good results. It was also observed that the $COD_{available}/P_{influent}$ ratio plays an important role in the development of a biomass capable of carrying out EBPR. A ratio between 10 to 15 mg of COD per mg of P is recommended. Although the $P_{released}/COD_{assimilated}$ ratio was low compared with what has been suggested in published research, this can be explained by the presence of GAOs.

Even though denitrification occurred in the process studied, it has been suggested that the organisms carrying it out were not—or were not exclusively—denitrifying PAOs but other organisms such as denitrifying GAOs. Given the probability of the presence of GAOs, it is recommended that the process be tested with another carbon source or that acetate be alternated with another carbon source in order to favour the selection of PAOs and denitrifying PAOs.

Despite the potential the process showed for EBPR and nitrate removal, there are three obstacles to overcome before implementing the process on a fish farm. First, the low phosphate concentration in PCC fish farm effluents would limit the biomass capable of EBPR and lead to diffusion problems through the biofilm. Second, a carbon source would be needed because fish farm effluents do not contain sufficient VFAs for PAO metabolism. The final obstacle is the operating complexity of the process studied, i.e., maintaining a $COD_{available}/P_{influent}$ ratio to guarantee an ideal concentration of substrate, managing the ST and water renewals, and having a second process for the disposal of the phosphate accumulated in the ST.

Conclusions

1. A SMBBR with a ST achieved phosphate removal (7.5 mg of P per litre of an influent spiked to 10 mg of P per litre) and nitrate removal (8.5 mg of N per litre of an influent containing 21 mg of NO_3-N per litre) from a fish farm effluent. To achieve phosphate and nitrate removal, 40 mg of COD per litre of acetate was consumed, i.e., 12% of what was added.
2. The ST was efficient in preventing nitrate from entering in contact with the substrate. The process thus favoured the selection of organisms synthesizing PHA from acetate.
3. The $COD_{available}/P_{influent}$ ratio played an important role in the development of a biomass capable of EBPR. A

ratio varying between 10 and 15 mg of COD per mg of P is recommended for the operation of the system studied.

4. The low $P_{released}/COD_{assimilated}$ ratio was attributed to the presence of organisms other than PAOs. It was also suggested that the denitrifying organisms were not exclusively denitrifying PAOs.

5. It was suggested that the complexity of the process, the need to monitor the substrate, and the low phosphate concentration found in most PCC fish farm effluents would make this process difficult to implement in full-scale fish farms.

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