The effect of substrate composition on the nutrient removal potential of sequencing batch reactors

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Abstract

Experimental results suggest that sequencing batch reactors (SBR) are not efficient for enhanced biological phosphorus removal from domestic sewage with low/medium organic carbon content when denitrification preferentially competes for available carbon. Total COD is not a meaningful parameter to reflect available substrate for N and P removal; COD fractionation and identification of the readily biodegradable COD fraction are required for an accurate assessment of system performance. The degree of soluble COD removal in the non-aerated phase is observed to be much higher than what may be calculated from stoichiometric relationships for N removal and P release, indicating the existence of competing mechanisms such as organic carbon storage by non-polyP bacteria under anaerobic conditions.

Introduction

It is well-known that enhanced biological phosphorus removal (EBPR) requires an anaerobic/aerobic sequence. In the anaerobic stage, phosphorus release is associated with storage of organic substrate within biomass. During the aerobic stage, excess phosphorus uptake takes place at the expense of stored organics which also serve as carbon source for the growth of micro-organisms which have the ability to accumulate polyphosphates (polyP bacteria).

The nature of the organic substrate available plays a key role for effective phosphorus removal which selectively requires the existence of short-chain fatty acids. It is now commonly agreed that the major function of the anaerobic stage is to generate the necessary fermented substrate which can be utilised by polyP bacteria. In all the kinetic models so far proposed, acetate is referred to as the sole external substrate which is taken up and stored as polyß hydroxybutyrate (PHB) during anaerobic conditions. The question of how a reducing power is created for PHB synthesis constitutes the main distinction between various biochemical models (Comeau et al., 1986; Wentzel et al., 1986; Mino et al., 1987). It has been shown that other short-chain fatty acids may also be used to a limited extent for the same purpose and in such cases, other polyhydroxyalkanoates are synthesised besides PHB (Satoh et al., 1992).

Currently, the sequencing batch reactor (SBR) technology is a well promoted and tested alternative with distinct advantages over the conventional activated sludge process. Basically, the SBR is a very simple system involving a single tank (Orhon and Artan, 1994). As it is temporally controlled, as contrasted to spatially controlled conventional continuous-flow processes, it offers a major advantage for the observation and the interpretation of different phenomena associated with the anaerobic/aerobic sequence of the EBPR process.

The magnitude of the available organic substrate and its readily biodegradable fraction is likely to have a decisive impact on the extent of EBPR from domestic sewage and such an impact can best be visualised within an SBR. EBPR may also be severely affected by simultaneous nitrogen removal which is often desired in systems treating domestic sewage. The presence of nitrate at the beginning of the anaerobic phase is detrimental to EBPR, since oxidised nitrogen will preferentially consume organic substrate for denitrification and this will create an anoxic phase before true anaerobic conditions can be sustained. In this case, the amount and the nature of substrate will have to be sufficient both for denitrification and PHB storage for EBPR.

The main objective of this experimental study was to investigate the effect of different substrate conditions and simultaneous nitrogen removal on the EBPR efficiency of an SBR system treating domestic sewage with a fluctuating organic content.

Materials and methods

The experimental work was carried out in a timer-controlled, laboratory-scale sequencing batch reactor with a total volume adjustable up to 8.8 ℓ. The reactor was equipped with both mechanical mixing and diffused aeration devices to create a sequence of anaerobic/anoxic and aerobic conditions. Wastewater feeding during the fill period was secured by an adjustable-flow Watson-Marlow type peristaltic pump and three different exit ports, each controlled by a solenoid valve, which provided discharge of the treated effluent.

The laboratory SBR unit was operated for around 200 d in four different consecutive runs. The operation was adjusted to four cycles a day (cycle time, $t_c = 6$ h) with the exception of run III where the cycle time was extended to 8 h (three cycles a day). Each cycle involved the regular consecutive sequence of anoxic/anaerobic fill and mixing phase, the aerated reaction phase and the settle/idle phase where the treated effluent was discharged. The operation conditions and the characteristics of the laboratory SBR unit are outlined in Table 1. The sludge age was controlled and the mixed liquor volatile suspended solids (MLVSS) concentration was kept constant for each individual run, by wasting the required amount of activated sludge, once a day, at the end of the aerobic period of the same cycle.

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TABLE 1
OPERATIONAL CONDITIONS AND CHARACTERISTICS OF SBR

Run	Duration (days)	Operational conditions						Reactor characteristics			
		m	t_F (h)	t_M (h)	t_A (h)	t_{S+I} (h)	t_C (h)	V_0 (ℓ)	V_F (ℓ)	V_0/V_F	V_F/V_T
Run I Start-up	76	4	2	2.5	2	1.5	6	4.4	2	2.2	0.31
Run II **Domestic wastewater**	28	4	2	2.5	2.5	1.0	6	3.3	2	1.65	0.37
Run III **Domestic wastewater**	36										
RunIII-1	14	3	2.5	3.5	3.5	1.0	8	3.3	2.6	1.27	0.44
RunIII-2	22	3	2.5	4.0	3	1.0	8	3.3	2.6	1.27	0.44
Run IV **Domestic ww + acetate**	54										
Run IV-1 50 mg·ℓ^{-1} acetate COD	10	4	2	2.5	2.5	1.0	6	3.3	2	1.65	0.37
Run IV-2 100 mg·ℓ^{-1} acetate COD	13	4	2	2.5	2.5	1.0	6	3.3	2	1.65	0.37
Run IV-3 300 mg·ℓ^{-1} acetate COD	13	4	2	2.5	2.5	1.0	6	3.3	2	1.65	0.37
Run IV-4 150 mg·ℓ^{-1} acetate COD	18	4	2	2.5	2.5	1.0	6	3.3	2	1.65	0.37

m: cycle number in a day; t_F: fill time; t_M: mix time; t_A: aeration time; t_{S+I}: settle and idle time; t_C: total cycle time;
V_0: initial volume; V_F: fill volume; V_T: total volume

During the start-up period (Run I) and Runs II and III, the system was fed with domestic sewage only, collected from the Greater Metropolitan Area of Istanbul. The relatively strong sewage from the Tuzla collection area was used for Run I and sewage from the Ataköy region was provided for Runs II and III. Run IV was conducted with four different feeding patterns, each using the domestic sewage from Ataköy, supplemented with different acetate concentrations.

The performance of the SBR system during each run was monitored continuously and evaluated at cyclic steady state conditions, in terms of daily measurements of the influent and effluent characteristics associated with a selected cycle. The evaluation also involved the fate of soluble COD, phosphate, ammonia and nitrate concentration profiles within a selected cycle, under steady state conditions characterising different runs.

All analyses were performed in accordance with *Standard Methods* (1989). The soluble fraction of COD and the other parameters were defined as filtrates through Whatman CF/C glass fiber filters with a effective pore size of around 1 μm. The same filters were also used to assess particulate components on a suspended solids (SS) or volatile suspended solids (VSS) basis. Phosphate and total phosphorus were measured using the ascorbic acid and persulphate digestion methods, respectively. Nitrate was reduced to nitrite in a cadmium reduction column and quantified with a colorimetric method. Ammonia was measured by means of the phenate method. The closed reflux titrimetric method was used for COD assessments.

Experimental results and evaluation

### Overall system performance

Start-up period

The study was started using a previously running SBR system. In this period, the reactor was fed with a synthetic wastewater basically prepared with Tryptose Soy Broth, (TSB) adjusted to an influent COD of 275 mg·ℓ^{-1} and a total P concentration of 8.3 mg·ℓ^{-1}. At steady state, the SBR was operated to sustain an MLVSS concentration of 1 070 mg·ℓ^{-1}, with a VSS/SS ratio of 0.76. Complete P removal was secured, resulting in a relatively high total P/MLVSS ratio of 8.3% for EBPR systems. The PO_4-P and COD profiles observed during the cyclic operation of the SBR system is illustrated in Fig. 1 (Tasli et al., 1997a).

During the 76 d start-up period, the SBR system was acclimated to domestic sewage provided from the Tuzla region in Istanbul, a relatively strong wastewater with average COD, TKN and total P concentrations of 420, 72, and 11 mg·ℓ^{-1} respectively. The system reached steady state after the first 50 d of operation: The MLVSS concentration increased from 1 070 mg·ℓ^{-1} to 2 880 mg·ℓ^{-1}, then stabilised at around 2 300 mg·ℓ^{-1}; the VSS/SS ratio dropped to 0.56 as a result of sewage with a relatively high concentration of inert suspended solids. Nitrification developed leading to a final NO_3-N level of 17 mg·ℓ^{-1}, with a corresponding TKN concentration of 4 mg·ℓ^{-1}. As far as EBPR was concerned, the effluent PO_4-P concentration first increased to 7 to 8 mg·ℓ^{-1} to finally level at 4.2 mg·ℓ^{-1}. The overall performance of the SBR during the start-up

TABLE 2
OVERALL PERFORMANCE OF THE SBR UNDER DIFFERENT OPERATING CONDITIONS

Run	Influent characteristics						Reactor characteristics				Effluent characteristics		
	Total COD mg·ℓ^{-1}	Acetate COD mg·ℓ^{-1}	TKN mg·ℓ^{-1}	TP mg·ℓ^{-1}	T.COD /TKN	T.COD/ TP	MLVSS mg·ℓ^{-1}	% P gP/ gVSS	VSS/SS	V_0/V_F	NH$_3$-N mg·ℓ^{-1}	NO$_3$-N mg·ℓ^{-1}	PO$_4$-P mg·ℓ^{-1}
Run I Start-Up	420	-	72	11	6	36	2 560		0.56	2.2	4	17	4.2
Run II **Domestic wastewater**	300	-	40	9	7.5	33	2 930	3.5	0.61	1.65	2	25	5.8
Run III **Domestic wastewater** RunIII-1	320	-	37	8.2	8.6	39	2 550		0.70	1.27	2	18.5	4.8
RunIII-2	325	-	35	9	8.6	39	2 175	3.5	0.76	1.27	3.5	17.0	3.5
Run IV **Domestic ww + acetate** Run IV-1	350	50	50	8	7	43	1 980		0.81	1.65	2	17	4.6
Run IV-2	490	100	46	7.5	10.6	65	2 240	3.4	0.83	1.65		20	4
Run IV-3	530	300	65	8	8	66	2 400		0.87	1.65		12	0.15
Run IV-4	360	150	49	9.6	7.5	38	2 040	5.3		1.65		12	1.0

Figure 1
COD and PO$_4$-P concentration profiles of the system fed with synthetic wastewater
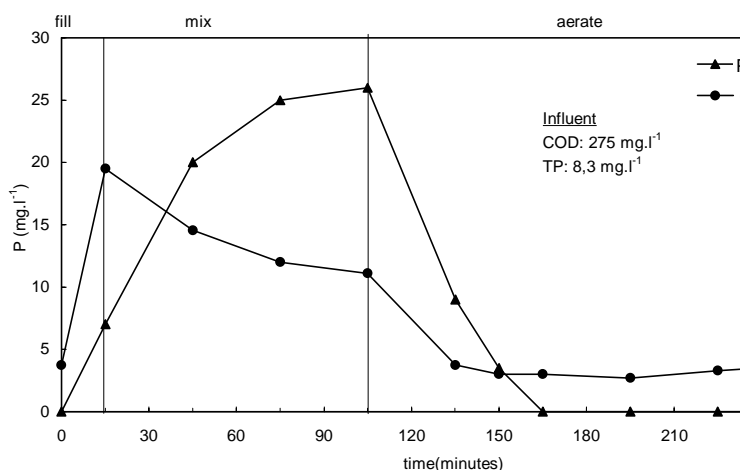
period is outlined in Table 2, along with the results associated with the other runs. The effluent quality obtained exhibits typical characteristics of a well-nitrifying system, with partial P removal.

Experiments with domestic sewage

The first part of the experimental study after start-up, involved investigating the EBPR potential of SBR using domestic sewage with a lower organic carbon content. The system was fed with domestic wastewater from the Ataköy area in Istanbul with an average COD content of 300 to 325 mg·ℓ^{-1} and a moderate COD/P ratio in the range of 33 to 39.

The study was conducted with the main objective to explore the impact of simultaneous nitrogen removal on EBPR, which is often desired in biological treatment processes. Moreover, simultaneous nitrogen removal can hardly be avoided, unless the system is operated at very low sludge ages. Unlike coventional continuous processes, an anoxic phase is inherently established in SBRs before

the true anaerobic phase, where the readily biodegradable COD fraction is consumed at the expense of available NO$_3^-$-N, reducing the PHB storage and the phosphorus release associated with EBPR. Therefore, the degree of P removal that can be achieved in nutrient removing SBR systems depends upon the delicate balance between organic carbon, nitrogen and phophorus levels and important operation parameters such as V_0/V_F ratio and the duration of the anoxic/aerobic conditions.

Experiments with domestic sewage were conducted in two stages. The SBR operation in the first stage (Run II), continued for 28 d, involving four cycles a day (6 h cycles). Each cycle included first a 2.5 h anoxic/anaerobic phase, where domestic sewage was fed during the first 2 h, a 2.5 h aerated phase, 0.5 h more than that applied in the start-up period, and a final 1 h settle, discharge/idle phase, a period kept relatively short because of good settling conditions. In this run, the initial SBR volume (V_0) was reduced to 3.3 ℓ; a fill volume (V_F) of 2.0 ℓ was maintained, resulting in a

V_F/V_T ratio of 0.37, and a V_0/V_F ratio of 1.65; the MLVSS concentration was slightly increased to 2 930 mg·ℓ^{-1} with a VSS/SS ratio of 0.6 ℓ. In this operation, the average effluent characteristics of NH_3-N = 2.0 mg·ℓ^{-1}, NO_x-N = 25 mg·ℓ^{-1} and PO_4-P = 5.8 mg·ℓ^{-1}, as listed in Table 2, indicate that both nitrogen and phosphorus removal efficiencies deteriorated.

Limited N and P removal in the SBR system may be attributed to either a lower readily biodegradable COD available at the beginning of the anoxic/anaerobic phase, or insufficient reaction time under anoxic/anaerobic conditions. To test the latter, the second stage operation (Run III-1), was adjusted to three cycles a day, thus creating a longer anoxic/anaerobic reaction time; the aeration phase was also set as 3.5 h. Accordingly, V_F was increased to 2.6 ℓ; V_0 was maintained as 3.3 ℓ, leading to V_F/V_T = 0.44 and V_0/V_F = 1.27. The MLVSS concentration was reduced to 2 050 mg·ℓ^{-1} with VSS/SS = 0.7. During the 20 d of operation, the influent quality was practically the same as that of the previous period though with a slightly lower TKN value and extending the anoxic/anaerobic phase provided, as shown in Table 2, only a slight improvement in nitrogen and phosphorus efficiencies; basically, the effluent NO_x-N dropped from 25 mg·ℓ^{-1} to 18.5 mg·ℓ^{-1}, inducing a moderate reduction in the corresponding PO_4-P concentration to 4.8 mg·ℓ^{-1}.

In the following 22 d (Run III-2), the anoxic/anaerobic phase was further increased to 4 h, keeping the three 8 h cycles, also increasing the MLVSS concentration to 2 840 mg·ℓ^{-1} and VSS/SS to 0.76. This change generated an effluent with only slightly lower NO_x-N and PO_4-P concentrations of 17 mg·ℓ^{-1} and 3.5 mg·ℓ^{-1}, respectively, providing a clear indication that the limiting factor was not the anoxic/anaerobic reaction time, but the amount of readily biodegradable COD to satisfy both the requirements of simultaneous denitrification and EBPR.

Experiments with domestic sewage supplemented by acetate

This part of the experiments (Run IV), was designed to investigate the impact of the available readily biodegradable COD on N and P removals, by supplementing the domestic sewage feed with different concentrations of acetate. During the entire run, a 6 h cycle time was adopted and the SBR volume fractions were maintained as V_0 = 3.3 ℓ, V_F = 2 ℓ and V_T = 5.3 ℓ as in Run II with domestic sewage corresponding to V_F/V_T = 0.37 and V_0/V_F = 1.65.

During the first two parts of the experiments (Runs IV-1 and IV-2), the acetate addition was adjusted to 50 and 100 mg·ℓ^{-1} COD equivalent of acetate, increasing the influent total COD concentration to 350 mg·ℓ^{-1} and 490 mg·ℓ^{-1} respectively. The corresponding COD/P ratios were calculated as 43 in the first part and 65 in the second part. As shown in Table 2, the desired EBPR performance could not be secured in both parts where the average effluent PO_4-P concentrations were measured as 4.6 mg·ℓ^{-1} and 4.0 mg·ℓ^{-1}. In Run IV-2, the P/VSS level was only 3.5%, a value that was also obtained in the previous experiments with only domestic sewage.

In the following part (Run IV-3), the acetate addition was further increased to 300 mg·ℓ^{-1} COD equivalent, with a corresponding total COD concentration of 530 mg·ℓ^{-1} and a COD/P ratio of 66. As outlined in Table 2, this part of the experiments was characterised by an effluent PO_4-P of 0.15 mg·ℓ^{-1} and NO_x-N of 12 mg·ℓ^{-1}. In the final part (Run IV-4), where the domestic sewage feed was particularly weak, the acetate concentration was lowered to 150 mg·ℓ^{-1} COD equivalent, resulting in a total COD of 365 mg·ℓ^{-1}; the same system performance could still be maintained with an effluent NO_x-N = 12 mg·ℓ^{-1} and a slightly higher PO_4-P of 1.0 mg·ℓ^{-1}. The results in Table 2 showed that the amount of readily biodegradable COD available in Run IV-3 with an acetate addition of 300 mg·ℓ^{-1} COD equivalent was more than what was required for a complete phosphorus removal and a nitrogen removal level that is potentially achievable with a pre-denitrification scheme simulated by the SBR operation. In this part, the SBR was observed to sustain a MLVSS concentration of 2 040 mg·ℓ^{-1}, with a P/VSS level of 5.3%, more compatible with EBPR.

The impact of denitrification on EBPR

PHB storage and phosphorus release during the first phase of the EBPR closely relates to the extent of readily biodegradable substrate available for this process. If simultaneous nitrogen removal occurs, this phase will initially sustain an oxidised nitrogen pool, (NO_x-N) acting as electron acceptor in the absence of dissolved oxygen and preferentially consuming the existing readily biodegradable substrate. Therefore, an anoxic phase is established before the true anaerobic conditions, significantly reducing EBPR, because the substrate fraction that is likely to be stored as PHB is diminished and the reaction time for acidification and formation of fermentation products (acetate), if present, is shortened. As a result, EBPR cannot take place or it can proceed only at a very reduced rate as reported in the literature (Satoh et al., 1994; Tasli et al., 1997b).

In this study the impact of nitrogen removal on EBPR was investigated by measuring and observing the concentration profiles of relevant parameters within a selected complete cycle during the steady state operation of the SBR under different conditions. The first cyclic experiment relates to Run II where the SBR was fed with domestic sewage alone in four 6 h cycles, with a V_0/V_F ratio of 1.65. On the day the experiment was carried out, the sewage characteristics were measured as COD = 220 mg·ℓ^{-1}, TKN = 40 mg·ℓ^{-1} and total P = 6 mg·ℓ^{-1}, a wastewater with a low organic content and consequently a low COD/N ratio of 5.75. The concentration profiles observed within one full cycle are illustrated in Fig. 2.

As shown in this figure, the SBR performed very poorly, producing an effluent with a NO_x-N concentration of 22.5 mg·ℓ^{-1}, and a PO_4-P concentration of 4.8 mg·ℓ^{-1}. During the aerated phase almost no phosphorus uptake occurs as evidenced by a PO_4-P drop from 5.5 mg·ℓ^{-1} to only 4.8 mg·ℓ^{-1}. The rational interpretation of this observation closely relates to nitrogen transformations taking place in the sequence of anoxic/aerobic phases of the cycle: The aerobic sludge age of the system was sufficient to secure complete nitrification and the NH_3-N content of the aerated phase was fully converted to NO_x-N. The V_0/V_F ratio in the SBR corresponds to the total recirculation ratio in continuous systems; consequently, a V_0/V_F ratio of 1.65 would potentially allow for an effluent NO_x-N concentration of around 13 mg·ℓ^{-1}, provided that the non-aerated phase secures full denitrification. For this experiment however, this was not the case, as the non-aerated phase remained fully anoxic and the NO_x-N profile persisted during the entire period, dropping from 22 mg·ℓ^{-1} to only 12 mg·ℓ^{-1}. Apparently, the COD content and its readily biodegradable fraction was not sufficient to consume all available oxidised nitrogen as electron acceptor and the polyP bacteria could not compete with denitrifiers for suitable biodegradable COD. As shown in Fig. 2, the relatively rapid initial decrease of the NO_x concentration in the first 30 min followed by a slower rate suggest that readily biodegradable COD (RBCOD) was used up within the first 30 min. leaving only slowly biodegradable COD (SBCOD) for further degradation using NO_x as the final electron acceptor. This may lead to conclude, in support of the experimental data that in the absence of RBCOD, no P release; no PHA stored and therefore no P uptake during the aerobic phase.

The same experiment was repeated for Run III-2; this run was

estimated to be better suited to nutrient removal, involving 8 h cycles and extended 4 h non-aerated periods. The experiment was also carried out with a more concentrated sewage of 390 mgO$_2$·ℓ^{-1}, TKN = 44 mg·ℓ^{-1} and total P = 10 mg·ℓ^{-1}. The results, given in Fig. 3, do not significantly differ from previous observation with regard to EBPR. Despite a substantially increased denitrification potential and decreased available nitrate due to lower recirculation ratio, the non-aerated period was still fully anoxic, sustaining at the end a residual NO$_X$-N concentration of 2.7 mg·ℓ^{-1}.

The two observations illustrated in Figs. 2 and 3, exhibit a striking example of RBCOD limitation for both N and P removal. A better interpretation of the results may be possible by looking at the COD fractions rather than the total COD of the sewage feed. It is not so much significant whether, on an overall scale, the total COD or the COD/N, COD/P ratios appear suitable for nutrient removal; the significant issue is rather the type of organic carbon available when and where it is needed and only COD fractionation can provide the related information. So far, extensive studies have been carried out on detailed characterisation of domestic sewage in Istanbul (Orhon et al., 1994; 1997). They indicate that around 15% of the total COD is non-biodegradable; of the remaining organic carbon content, only 10% is readily biodegradable and the bulk (around 75%) is of slowly biodegradable nature, an observation quite in accordance with the findings previously reported in the literature for domestic sewage. As regards the two cyclic observations, the total COD feed of 300 to 400 mg·ℓ^{-1} is likely to include a readily biodegradable fraction of only 30 to 40 mg·ℓ^{-1}, clearly insufficient to satisfy denitrification requirements and the hydrolysis rate of the slowly biodegradable components appears to be too slow to meet the additional demand.

The third experiment of the series was carried out during Run IV-3, where the readily biodegradable COD fraction of the domestic sewage feed was supplemented by a 300 mg·ℓ^{-1} COD equivalent of acetate addition (Fig. 4). The feed characteristics associated with the experiment were measured as total COD = 540 mg·ℓ^{-1}, TKN = 65 mg·ℓ^{-1} and total P = 7.5 mg·ℓ^{-1}. The results obtained, plotted in Fig. 4, show that with this level of organic carbon available, the NO$_X$-N was entirely consumed during the first hour of the non-aerated period; the system was then able to sustain true anaero-
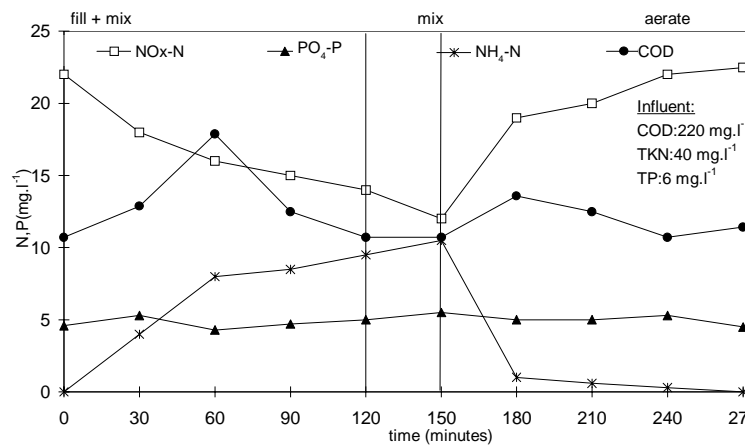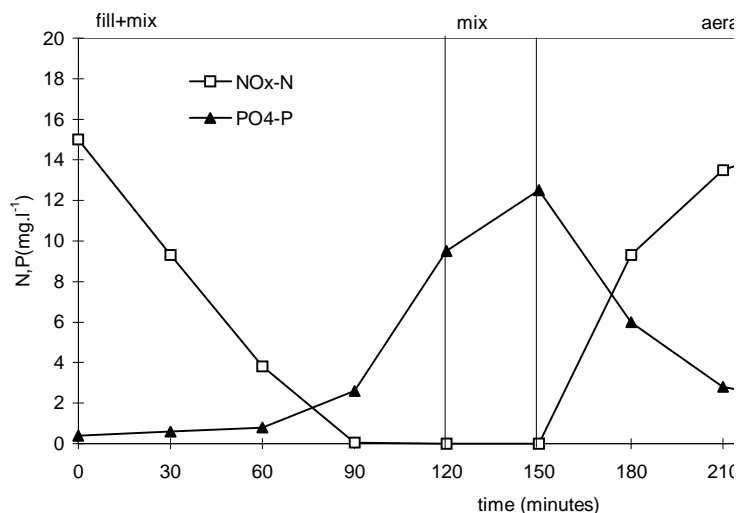

Figure 2
COD, PO$_4$-P, NO$_x$-N and NH$_4$-N concentration profiles of Run II fed with domestic wastewater
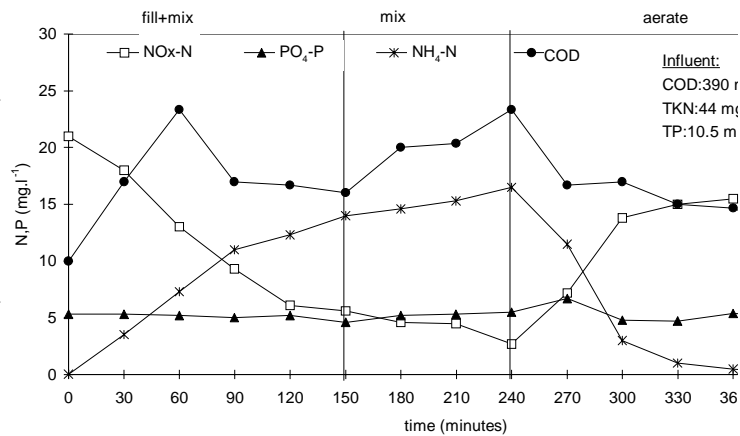
Figure 3
COD, PO$_4$-P, NO$_x$-N and NH$_4$-N concentration profiles of Run III-2 fed with domestic wastewater
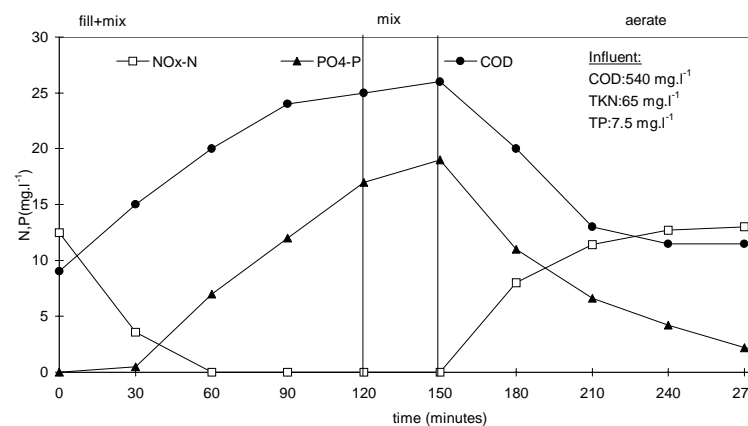
Figure 4
COD, PO$_4$-P, and NO$_x$-N concentration profiles of Run IV-3 fed with domestic wastewater with 300 mg·ℓ^{-1} COD equivalent acetate addition

Figure 5
PO$_4$-P, and NO$_x$-N concentration profiles of Run IV-4 fed with domestic wastewater with 150 mg·ℓ^{-1} COD equivalent acetate addition

bic conditions resulting in a PO$_4$-P concentration of 19 mg·ℓ^{-1} in the total reactor volume due to phosphorus release. The subsequent P uptake in the aerated phase reduced the effluent PO$_4$-P level to 1.0 mg·ℓ^{-1}.

The last experiment reflects the typical SBR operation during Run IV-4 where the acetate addition was lowered to 150 mg·ℓ^{-1}; the feed had a total COD content of 300 mg·ℓ^{-1}, together with a TKN of 75 mg·ℓ^{-1} and a total P of 8 mg·ℓ^{-1}. The results are given in Fig. 5.

It is interesting to note that although the total COD available was lower than that of the second experiment conducted with domestic sewage alone (Fig. 3), the difference in its composition, namely the extent of its readily biodegradable fraction, increased with acetate addition, was instrumental for a total NO$_x$-N depletion in 1.5 h; the remaining 2 h anaerobic phase was sufficient for an efficient EBPR with a final PO$_4$-P concentration of 1.1 mg·ℓ^{-1} observed at the end of the aerobic phase. Figs. 4 and 5 jointly showed that P release rate increases after NO$_x$ is depleted, supporting the previous observations on simultaneous P release and anoxic P uptake reported in the literature (Artan et al., 1998; Kuba et al., 1993; Gerber et al., 1987).

COD balance in the anoxic/anaerobic phase

As observed in the previous section, the magnitude of the available RBCOD plays a key role in N and P transformations taking place during the non-aerated phase. From a conceptual standpoint, COD uptake within the non-aerated phase in an SBR can only be attributed to NO$_x$-N consumption and to PHB storage with P release, either occurring simultaneously or sequencially. Current mathematical models do not allow for heterotrophic growth under anaerobic conditions for P-removing systems (Gujer et al., 1995). COD removal is preferentially in the form of readily biodegradable/soluble COD for N removal and definitely in the form of fermentation products (acetate) for PHB storage. These COD fractions may be present in the influent feed or they may also be generated through hydrolysis of particulate and soluble slowly biodegradable organics under anoxic or anaerobic conditions. Theoretically, COD requirements for both N removal and P release can be calculated from stoichiometric relationships: For N removal, the corresponding COD requirement can be calculated from the following expression:

$$\Delta COD_N = \frac{\Delta N \, 2.86}{1 - Y_{NH}}$$

where:

Y$_{NH}$ is observed heterotrophic yield (g COD. g^{-1}COD)

Similarly, current models define a coefficient, Y$_{PO4}$, reflecting the stoichiometric relationship between P release and PHB storage under anaerobic conditions (Gujer et al., 1995). The suggested value for Y$_{PO4}$ is 0.4 g P/g COD, and consequently, the amount of COD removed from solution for PHB storage may be calculated as:

$$\Delta COD_P = \frac{\Delta P_{released}}{Y_{PO_4}}$$

The above considerations provide a simple way of calculating the theoretical COD consumption in the anoxic/anaerobic phase on the basis of NO$_x$ consumed and P released. The COD balance was tested using the concentration profiles of soluble COD, N and P obtained by means of the cyclic measurements within Runs II, III-2 and IV-3. The experiment associated with Run II was started with a soluble COD of 120 mg·ℓ^{-1}. From the data presented in Fig. 2, the soluble COD removal at the end of the 2 h fill phase was 90 mg·ℓ^{-1}. As previously mentioned, there was no noticeable P release and denitrification during the same phase was very limited, as the NO$_x$-N concentration could be reduced from 22 mg·ℓ^{-1} in the initial volume, V$_0$, of 3.3 ℓ to 12 mg·ℓ^{-1} in the final volume, V$_T$, of 5.3 ℓ, corresponding to 4.5 mg of NO$_x$ removal per ℓ of wastewater treated; this level of electron acceptor consumption could only account for around 17 to 18 mg of COD depletion per ℓ of wastewater treated. The soluble COD input for the same experiment in Run III-2 was 200 mg·ℓ^{-1} and accordingly N removal in the fully anoxic non-aerated phase was increased to 21 mg per ℓ of wastewater treated, with a COD equivalent of around 80 mg per ℓ of wastewater treated. This value is still lower than 130 mg·ℓ^{-1} soluble COD decrease observed at the end of the same period.

The cyclic observation associated with Run IV-3 was started with a much higher soluble COD concentration of 400 mg·ℓ^{-1}, due to acetate addition. At the end of the non-aerated phase, soluble COD removal was computed to reach 296 mg per ℓ of wastewater treated. As shown in Fig. 4, this phase involved a sequence of two distinct anoxic and anaerobic periods: In the first two hours complete denitrification took place with an NO$_x$-N removal of 20.6 mg per ℓ of wastewater treated and the remaining anaerobic period induced a rather intense P release of 43 mg per ℓ of wastewater treated. From the basic stoichiometric relationships, the corresponding soluble COD consumption was calculated as 82 mg per ℓ of wastewater treated for N removal and 108 mg per ℓ

of wastewater treated for P release, the total of which being significantly lower than 296 mg·ℓ^{-1} observed in the same period.

It should be noted that the above calculations are very conservative, mainly because they do not include the impact of the RBCOD generation through hydrolysis (Orhon and Artan, 1994; Gujer et al., 1995) and soluble inert COD generation as residual metabolic products (Orhon et al., 1989); for domestic sewage, the level of soluble residual microbial products may be assumed as around 5% of the total biodegradable COD removed (Orhon et al., 1994; 1997). Hydrolysis of particulate COD components should be expected to proceed at a higher rate. Therefore, the discrepancy between theoretical COD balance derived from stoichiometric relationships involving COD, N and P and the removal of soluble biodegradable COD is likely to be more pronounced than what may be calculated from measured soluble COD profile in the course of the anoxic/anaerobic phase.

These experimental results suggest the existence of a simultaneous microbial process taking up available organic carbon and thus severely competing with denitrification and P removal. Storage of the soluble organic carbon before biosynthesis, by heterotrophic biomass under aerobic and/or anoxic conditions, as advocated in recent experimental and modelling studies (Majone et al., 1998; Gujer et al., 1998), may be a likely explanation for the observed results. This is an indication that current modelling concepts attributing COD removal during the non-aerated phase to only NOx-N removal and PHB storage/ P release are not substantiated; this assumption may at times be misleading as it tends to overestimate the N and P removal potential of the biological system. In fact, the anoxic storage of soluble substrate may significantly delay the nutrient removal mechanism as it would move the oxidation of the stored materials to the aerobic phase instead of in the anoxic phase.

Conclusions

The results and evaluations of this study may be summarised as follows:

- The main difference between continuous-flow and SBR activated sludge systems for N and P removal is that an anoxic phase is inherently established before truly anaerobic conditions leading to PHB storage and P release. Denitrification preferentially consumes available organic carbon, thus blocking biological processes related to EBPR. Therefore, as the experimental findings of this study confirm, SBR is not likely to perform efficiently with respect to EBPR, for domestic sewage with low/medium RBCOD content.

- As the process efficiency should be directly related to RBCOD availability in the influent wastewater, total COD or ratios based on total COD, such as COD/N, COD/P are incapable of reflecting the available readily biodegradable organic carbon potential within the non-aerated phase. Experimental results showed that while domestic sewage with a total COD content of 390 mg·ℓ^{-1} could not even satisfy the organic carbon demand of denitrification, full N and P removal was restored when a mixture of domestic sewage and acetate with a COD equivalent of only 300 mg·ℓ^{-1} was used. COD fractionation and identification of the readily biodegradable COD fraction are required for an accurate assessment of system performance.

- The degree of soluble COD removal in the non-aerated phase was observed to be much higher than what could be calculated from stoichiometric relationships for denitrification and P release, indicating the existence of competing mechanisms

such as anoxic storage of readily biodegradable substrate and organic carbon storage by non-polyP bacteria under anaerobic conditions. Therefore it would be misleading to predict or design a nutrient removal system on the basis of the theoretical COD balance, as the corresponding estimation of the available organic carbon is likely to be too optimistic to satisfy N and P requirements.

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