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ACIDOGENIC ACTIVITY: PROCESS OF CARBON SOURCE GENERATION FOR BIOLOGICAL NUTRIENT REMOVAL

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ABSTRACT

In this study, a sequencing batch reactor (SBR) connected with a two step anaerobic digestion system is proposed in order to investigate the possibility of simultaneous C, N and P removal from wastewater. The system was studied using synthetic wastewater. In this system, the effluent of nitrate from the SBR reactor is added to the acidogenic reactor influent. Nitrate elimination and VFA production are then achieved together in the acidogenic reactor. The performances of three lab-scale reactors, operated for C, N and P biological removal are analyzed. The removals of TOC, TN and TP-PO_4 were greater than 96%, 75% and 86%, respectively. The results show that the combination of anaerobic digestion in two step-SBR treatment is effective for simultaneous C, N and P removal. The benefits from this process are the saving of carbon source for denitrification and phosphorus removal. Reactor arrangement made possible the existence of zones where the different bacterial populations involved could coexist. Complete denitrification occurs in acidogenic reactor and hence the methanogenic activity is not reduced nor inhibited by N-NO_3 presence, allowing greater TOC removal. A stable P-release and P-uptake took place after coupling of the three reactors. Furthermore, a fast settling, compact sludge is generated in the SBR with the operational conditions applied. © 1999 Published by Elsevier Science Ltd on behalf of the IAWQ. All rights reserved.

KEYWORDS

Biological Nutrient Removal (BNR); denitrification; nitrification, P-release, P-uptake, Sequencing Batch Reactor (SBR), Total Organic Carbon (TOC).

INTRODUCTION

Biological phosphorus removal processes are widely studied to prevent water pollution caused by eutrophication. In order to enhance biological nutrient removal (BNR) and improve the process operation, acid fermentation of primary sludge has been employed in some full-scale plants for BNR in conventional flow-through treatment.

The VFA produced during the first step of anaerobic digestion (hydrolysis and acidogenesis) can be used in BNR as carbon source (Rustrian *et al.*, 1997a). Moreover, acidogenic reactors have denitrification potential (Bernet *et al.* 1996). Denitrification occurs in an acidogenic reactor, so as to achieve simultaneous VFA

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production and nitrate elimination, a system that could be applied to organic carbon and nitrogen removal from food-processing wastewaters (Rustrian *et al.*, 1997a).

In this paper, a two-stage anaerobic digestion system coupled with a SBR system was developed. Although most studies have been carried out by continous flow reactors. The SBR system is well adapted to the arrangement of operational sequence that provides anoxic and anaerobic stirring, which promote denitrification and biological phosphorus release prior to the aeration sequence. The system consisted of an acidogenic and methanogenic unit and a SBR for BNR. The fermented wastewater (containing the VFAs), is conveyed to a BNR (SBR), and to a methanogenic unit where phosphorus and carbon respectively are removed efficiently. Synthetic wastewater was used for all experiments. Hence, the differences between the proposed system and traditional process are: VFA are produced by acidification of liquid influent and not by sludge fermentation, N-NO_3 recycling is proposed from SBR at acidogenic reactor (denitrification occurs in the acidogenic reactor); acidogenic outlet is the methanogenic inlet; SBR is fed with a mixture of acidogenic and methanogenic outlet. Finally, solids recirculation is minimized by settling of methanogenic and SBR outlets before recycling.

The objective of the process is to establish a C, N and P elimination activity and to obtain better steady state conditions than with continuous installations.

METHODS

Apparatus and operation

The system depicted in Figure 1 is divided into two parts: two-step anaerobic digestion and a sequencing batch reactor (SBR).

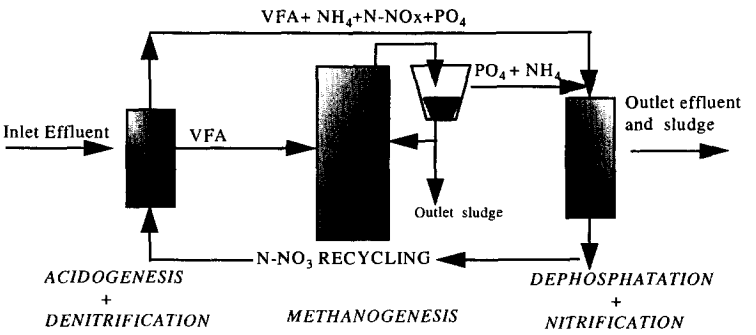

Figure 1. C, N and P removal system.

Acidogenic and methanogenic systems are the lab-scale continuous stirred reactors. Water (thermostatically regulated at 35°C) was circulated through a jacket on each reactor. Operational conditions are summarized in Table 1. A synthetic feed (Appeldoorn medium, Appeldoorn *et al.*, 1992) was used during all experiments and its composition was based on glucose, yeast extract and peptone as organic substrates to simulate the readily biodegradable fraction of a municipal wastewater (COD was adjusted by glucose increasing). The methanogenic reactor was fed with the acidogenic reactor outflow.

A laboratory-scale SBR consisted of a 2 l reactor (Biolaffite) in which the different environmental conditions (anaerobic/aerobic) were applied. The system was fed with Appeldoorn medium (Appeldoorn *et al.*, 1992) and operated in four 6-hour cycles each day. The operational conditions are shown in Table 1. The mixed liquor volume in the reactor at the end of the filling period was 1.5 l; 0.5 l of clarified supernatant were drawn after settling and 0.5 l of influent were added to the biomass remaining in the reactor from the previous cycle. The sludge age of the system was controlled at about 12 days by drawing the appropriate amount of sludge at the end of the aerobic phase in a cycle.

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Table 1. Working conditions of SBR and two-step anaerobic digestion system

Operative conditions	ACIDOGENIC Start-up and Run 1	Run2	METHANOGENIC Start-up and Run 1	Run2	SBR Start-up	Run 1	Run2
Feed characteristics :					COD : P 100 : 2	100 : 2.3	100:2.4
COD ($mg.l^{-1}$)	5222	5274	2460	2390	852	690.1	810.16
TOC ($mg.l^{-1}$)	2178	2312	1423	1460	PO_4-P* 17.08	16.19	19.6
VFA_{total} ($mg.l^{-1}$)	0	0	1.38	1.74	KH_2PO_4* 20		
VSS ($mg.l^{-1}$)	0.68	0.55	3.0	3.5	K_2HPO_4* 47		
Operational Conditions					SBR Cycle phase during : 6.0 H		
Working volume (l)	1.0		9.0		2.0		
Temp (°C)	35		35		30		
PH	6.5		7.5		7.5		
Mixing (rpm)	450		500		300		
HRT (d)	0.52		6.4		0.75		
Bv ($gCOD.l^{-1}.d^{-1}$)	10		0.75		Aereation (v.v.m) 1.0		
SBR Configuration cycle	Fill 8min,		anaerobic 2h30min,		aerobic 3h, settling 15min , draw 6min		

* ($mg.l^{-1}$)

Analytical methods

During all experiments, the characteristics of the influent and the effluent were measured routinely. Supernatants of samples were centrifuged at 12 000 g for 15 minutes with a Beckman J2-21 M/E centrifuge, then analysed for *COD, TOC*, N-NOx, *TKN*, N-NH_4 and VFA concentrations using Standard Methods (APHA, AWWA, WPFC). Total suspended solids were also determined, as well as gas composition and pH, in order to estimate the performances in the process.

Phosphate, nitrate and nitrite were analysed by ion chromatography system, using conductivity detection (Dionex-100). Separation and elution of the anions were carried out on Ion Pack AS4A analytical column using a carbonate-bicarbonate eluant and sulphuric acid regenerant. Integration was done using a Chromjet integrator (Spectra-Physics). Volatile fatty acids (*VFA*) analyses were done by gas chromatography with a flame ionisation detector (Chromapac CP 9000) coupled with an integrator (Shimadzu CR 3A). Total organic carbon (*TOC*) was titrated by *UV* oxidation with a Dohrman DC 80 apparatus. Gas was analysed by gas chromatography with Shimazdu GC 8A apparatus with argon carrier using catharometer detector. The chromatograph was coupled to a Shimadzu CR 3A integrator.

Calculations. Acidification degree (%), and the rate of product formation (rp) was calculated in accord with Dinopoulu *et al.*, 1998.

Experimental procedure

The reactors were first operated separately in order to have good acidogenic, methanogenic and dephosphatation steady state activities. They were then coupled according to the flow sheet depicted on Figure 1. The SBR operation is divided into three periods (Table 1) SBR start up, Run 1 to test the C/P and Run 2 for the C/N/P flux.

SBR cycle measurements were monitored periodically to examine the kinetics of phosphorus, VFA and nitrate in the anaerobic/aerobic phases. Soluble components (TKN, N-NH_4, P-PO_4, N-NO_3, N-NO_2, COD, TOC, sugar and VFA) gas composition and solid contents in each reactor were analyzed routinely.

RESULTS AND DISCUSSION

Unconnected reactors, start up period

Two-Phase Anaerobic Digestion System. In Table 2, the influent and effluent mean values for the start-up periods are shown. The acidogenic reactor was inoculated with digested sludge from a municipal treatment plant at a concentration of 5 g $VSS.l^{-1}$ and filled with synthetic wastewater at a COD concentration of 5.22

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$g.l^{-1}$. After inoculation, the reactor was operated in batch mode for 48 h, after which continuous feed was started. A step increase of the organic loading rate in conjunction with a step decrease of the hydraulic retention time was applied. Steady-state conditions at an HRT of 0.52 days (12.2 h) and organic load rate equal to 10 g $COD.l^{-1}.d^{-1}$ were reached after 50 days operation with 28 % of acidification degree and 1.4 g VFA. l^{-1}. $P\text{-}PO_4$ concentration in acidogenic feed composition was modified, KH_2PO_4 added was only 20 $mg.l^{-1}$ (before the coupling to have the same phosphate ($P\text{-}PO_4$) concentration of SBR synthetic feed in the mixed liquor of acidogenic-methanogenic effluents). In the last experiment (run 2), $(NH_4)_2\ SO_4$ was added as nitrogen source (295.88 $mg.l^{-1}$) to study the CNP ratios in the system.

Table 2. Start-up of anaerobic digestion in two steps, experimental results. Effluent characteristics, mean values of measured parameters

Parameters	Influent	Acidogenic Effluent	Methanogenic Effluent
TOC ($mg.l^{-1}$)	2178	1423	198
COD $mg.l^{-1}$)	5222	2460	236
VFA_{total} ($mg.l^{-1}$)	0	1380	130
Acidif. Degree (%)	--	27.23	--
rp (g $VFA.l^{-1}.h^{-1}$)	--	0.26	--
TOC removal (% of TOC inlet)	--	--	86
Biogas (l CH_4.g DCO $removal^{-1}$)	--	--	0.36

The methanogenic reactor was inoculated with sludge from an anerobic lagoon treating wine distillery effluent. Methane production of 0.364 l CH_4. g COD $consumed^{-1}$ and 86% COT removal are obtained (Table 2). The methanogenic reactor was fed continuously with 75% of total acidogenic effluent. After an acclimatization and steady-state period of 30 days, the methanogenic outflow was mixed with the acidogenic reactor outflow in proportion 2:1 v/v. This liquor was used to feed the SBR system in run 1 and 2, when the system was coupled.

During SBR start-up, a laboratory SBR system was operated for nutrient removal to investigate the stability of phosphorus removal capacity. The SBR system was inoculated with sludge from an EBRS treating a domestic wastewater at a concentration of 4.5 g $VSS.l^{-1}$. After inoculation, the reactor was operated in 6 h cycles (Table 1).

The experimental results obtained in the SBR start-up period are reported in Table 3. In these operating conditions, P-release and P-uptake were stablised and only denitrification activity was observed. The performance on P removal was affected by the anaerobic solids retention time (SRT) given by the length of anaerobic step. After 110 days of operation, growth of phosphorus accumulating organisms has been established. Concentration profiles indicate the possibility for complete phosphorus removal during the aerobic cycle of the process. It was observed, however, that phosphorus removal rate was not stable. Excellent settling sludge was obtained.

A suitable DCO : P ratio in the influent is necessary to ensure P-uptake. Significant release of phosphate was achieved in the anaerobic step in the start-up period. Nevertheless, there did not appear to be any relationship between the amount of phosphate released and the overall phosphorus removal.

In previous experiments with pure cultures of bio-P bacteria, phosphorus released under anaerobic conditions was not always related to the amount of VFA or phosphorus consumed. Moreover, P-uptake was found to be independent of phosphorus release rates (Rustrian *et al.*, 1997b). This indicates that the release of phosphate may be a signal of the establishment of suitable anaerobic conditions, rather than an essential intermediate in the process (Davelar *et al.*, 1978).

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Table 3. SBR start-up, experimental results. Effluent characteristics, mean values of measured parameters.

	TOC ($mg.l^{-1}$)	COD ($mg.l^{-1}$)	Acetate ($mg.l^{-1}$)	Glucose ($mg.l^{-1}$)	P-PO_4 ($mg.l^{-1}$)	N-NOx ($mg.l^{-1}$)	MLVSS ($g.l^{-1}$)
Influent	469.0	852.0	300.0	92.0	17.08	3.07	3.92
end of ANA	15.4	20.4	0	0	38.12	1.80	
end of AER	7.6	11.0	0	0	6.21	1.80	

Connected system

Two different operating conditions were tested on the connected system proposed (Table 1, runs 1 and 2).

Run 1. In this run, the C and P fluxes were studied. Acidogenic feed was adjusted in P-PO_4 concentration according to Appeldoorn medium (Appeldoorn et al. 1992). A 20-fold concentration medium (100 ml) was diluted every day with SBR effluent (1900 ml). Feed was changed one week before the connection of the system. Acidogenic reactor reached a 37% degree of acidification with 1.56 g VFA. l^{-1} (1.04 g of which being acetate). Performances in the acidogenic, methanogenic and SBR effluents are shown in Table 4.

Table 4. Run 1. System performances with C/P feed.

	TOC ($mg.l^{-1}$)	P-PO_4 ($mg.l^{-1}$)	MLVSS ($mg.l^{-1}$)	N-NOx ($mg.l^{-1}$)
Influent	2466	16.07	0	0.22
Acidogenic Effluent	2010	13.10	0.55	0
Methanogenic Effluent	203	9.28	3.50	0.56
SBR Influent	651	16.20	0.20	0.32
SBR Effluent	85	5.54	4.20	0

The methanogenic reactor showed 96% TOC elimination and 0.296 l CH_4. g COD $removed^{-1}$.

The SBR system was fed with a mixture of acidogenic (1/3) and methanogenic (2/3) effluents. VFA in the acidogenic effluent represented 60% of the inlet TOC. Only 8% of the influent TOC remained after methanisation, and 3.4% at the end of the aerobic step in the SBR. The P-PO_4 concentrations at the end of anaerobic and aerobic steps during run 1 and 2 are shown in Figure 2.

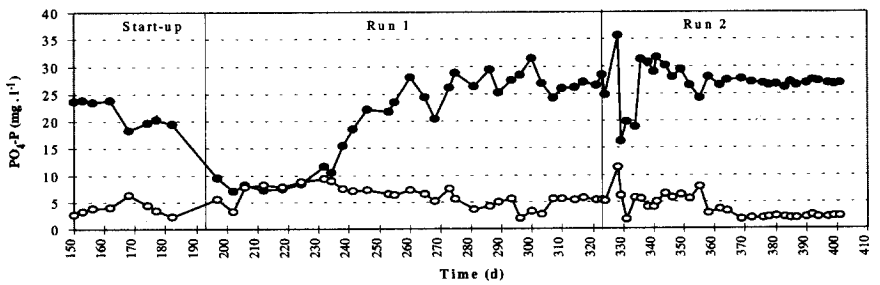

Figure 2. P-PO_4 amounts at the end of anaerobic (●) and aerobic (○) steps for the SBR cycles during Runs 1 and 2.

The P-removal activity decreased during the next 40 days of connecting operations. P-uptake was more stable than P-release in this period.

The limited availability of soluble ammonia can explain the limited amount of nitrate measured in the effluent (0.34 mg N-$NO_3.l^{-1}$ on average).

Run 2. The acidogenic feed was modified. A 20-fold concentration medium (100 ml) was diluted every day with SBR effluent (1900 ml) and NH_4 was added (295.88 $mg.l^{-1}$ $(NH_4)_2SO_4$) to acidogenic media composition (Appeldoorn et al. 1992) in order to study the C, N, and P fluxes in the system. SBR was fed

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with a mixture of acidogenic (1/3) and methanogenic (2/3) effluents. Best acidogenic reactor performances were obtained in this run. 56% of acidification ; 2.5g $VFAt_{otal}.l^{-1}$ with 1.45 $g.l^{-1}$ acetate was maintained. Ammonification was not observed, NH_4 concentrations in the acidogenic effluent were low. Nitrification took place in the SBR aerobic period. 12.64 $mg.l^{-1}$ $N\text{-}NO_3$ were found in the SBR effluent, and it was recycled to the acidogenic reactor where it was efficiently denitrified. This amount plus the soluble ammonia added at feed medium, were readily oxidized in the system (Table 5).

Methanogenic reactor performances were as good as in the first run. 88% of the TOC was eliminated, methane was produced at high rates: 0.302 ml CH_4.g $CODremoved^{-1}$.

The mean parameter values of $P\text{-}PO_4$ present at the end of anaerobic and aerobic step in the SBR, are resumed in Table 5. Performances were modified during the first 20 days after operating conditions were changed. P-release decreased. After about 2 months of operation, the P-removal activity was recovered. Significant anaerobic P-release and 86% of P-removal was obtained.

Table 5. Run 2. System performances with C/N/P feed. Mean experimental parameters values.

	TOC ($mg.l^{-1}$)	TOC-VFA ($mg.l^{-1}$)	$P\text{-}PO_4$ ($mg.l^{-1}$)	MLVSS ($g.l^{-1}$)	NTK ($mg.l^{-1}$)	$N\text{-}NO_3$ ($mg.l^{-1}$)	$N\text{-}NH_4$ ($mg.l^{-1}$)	N total ($mg.l^{-1}$)
Influent	2200	0	19.6	0	75.04	10.86	36.4	85.9
Acid. Effluent	1460	1056	16.9	0.5	32.47	0	8.4	32.47
Methan.Effluent	220	0	30.63	3.8	50.96	0	15.12	50.96
SBR Influent	710.4	698.3	26.13	0.16	42.56	0	11.2	42.56
SBR Effluent	80	0	2.79	4.7	8.72	12.64	0	21.36

A mass balance was performed with the mean parameters values obtained into the start-up periods and runs 1 and 2 for each reactor. Their performances are summarized in Table 6.

Table 6. Reactor performances obtained during start-up and Runs 1 and 2.

		START-UP	RUN 1	RUN 2
ACIDOGENIC REACTOR	Acidogenic Degree (%)	28.00	37.10	56.70
	VFA ($g.l^{-1}$)	1.40	1.60	2.50
	Acetic acid ($g.l^{-1}$)	0.90	1.10	1.90
	rp ($g.l^{-1}.h^{-1}$)	0.26	0.30	0.48
METHANOGENIC REACTOR	TOC removal (%)	86.00	90.00	88.00
	Biogas Yield ($mlCH_4.g\ COD^{-1}removal$)	0.36	0.29	0.30
CONNECTED SYSTEM	C* (% of TOCinlet)		96.00	96.00
	N* (% of TNinlet)			75.00
	P* (% of $TP\text{-}PO_4$ inlet)		66.00	86.00

* Removal

Carbon Flux. In the acidogenic reactor, the predominant fermentation product was acetic acid. The increase in VFA production observed in run 2 indicates that ammonia nitrogen added favoured acetic formers bacteria. The degree of acidification achieved during the current study varied between 28 and 57% of initial COD concentration. VFA in the acidogenic effluent is adequate to maintain the required level of carbon source flux in the system. The gas produced in the methanogenic reactor, was composed mainly of methane (75% on average) and carbon dioxide (22%). At steady-state, TOC removal efficiency varied from 86 to 90%. Finally, in the SBR, the VFAs in the feed were consumed in the first hour of the anaerobic step. Influent TOC varied between 651 $mg.l^{-1}$ (Run 1) and 710 $mg.l^{-1}$ (Run 2). It contained 67% and 79% of acetic acid, respectively.

Results obtained in run 1 indicated that 96% of inlet TOC is removed. 18% is consumed by the acidogenic reactor, 73% is eliminated by methanogenic reactor and 5% by the SBR.

In run 2, the amount of TOC removal for the system is constant (96%), but the acidogenic reactor consumption is about two times higher (34% of inlet TOC). This increase could be explained by the denitrification activity developed in this reactor ; methanogenic reactor removed 58% of TOC inlet and SBR

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4%. The carbon flux established is appropiate to supply the different populations involved in the C, N, P removal and their activities. The global TOC eliminated by the system fed with or without ammonia nitrogen was also satisfying.

Nitrogen Flux. In the first run, nitrogen source was limited in order to enhance the bio-P population activity. In run 2, when ammonia was fed, the following behaviour was observed. In the acidogenic reactor, ammonia was assimilated at a rate of 74.2 mg $N\text{-}NH_4$. g $VSS^{-1}.d^{-1}$, and the denitrification rate was 44.4 mg $N\text{-}NO_3$.g $VSS^{-1}.d^{-1}$. Hence, low nitrate concentrations could be eliminated in the anaerobic reactors, resulting in higher TOC reduction efficiency. These results are in agreement to those of Akunna *et al.,* 1992. It is due to the extra TOC requirements for N-NOx reduction and by best methanogenic activity. The amount of ammonia increased in the methanogenic reactor (+ 7 $mg.l^{-1}$). This may be due to the lysis of acidogenic cells brought with the influent, all the more since no nitrate was detected in the inlet or in the outlet of the reactor.

In the SBR, ammonia was constant under the anaerobic step and it dropped almost to zero at the end of aerobic step with nitrification. Nitrification rate of ammonia nitrogen was 2.75 mg.g VSS^{-1}. d^{-1}.

The average of total N eliminated by the system was as high as 75%, 62% by the acidogenic reactor and 13% by the SBR. Nitrate nitrogen was denitrified in early anaerobic conditions.

Phosphorus Flux. The acidogenic and methanogenic reactors showed a phosphorus removal as high as 18.5 and 24 %, respectively, in the first run. In the second run, acidogenic reactor $P\text{-}PO_4$ elimination was also 14% inlet $P\text{-}PO_4$, but methanogenic effluent $P\text{-}PO_4$ increased by 11 $mg.l^{-1}$. The $P\text{-}PO_4$ and the $N\text{-}NH_4$ increase observed could be due to lysis of the cells during the settling of methanogenic effluent. Hence, more $P\text{-}PO_4$ is present in the SBR feed.

In the SBR, phosphorus removal in the first run was over 66%. It was higher in the second run (86% of $P\text{-}PO_4$ inlet). This increase could be explained by the increase of COD : P ratio. The required microorganisms were present in the inoculum used. But, in suspended systems, the length of the lag phase during recovery is directly proportional to the length of the previous starvation phase (Davelar *et al.* 1978). Gradual changes can thus be handled easier by a microbial community than abrupt changes. The $N/P\text{-}PO_4$ ratio applied in run 2 was 3.82 for the system and 1.63 for the SBR, and 86% and 90 % of $P\text{-}PO_4$ removal was reached, respectively. Yong *et al.* (1996), obtained 87% of $P\text{-}PO_4$ removal using a $N/P\text{-}PO_4$ ratio of 4 with a single reactor combining anaerobic and aerobic conditions.

The studies conducted by Menar and Jenkis (1970) indicate that activated sludge can remove 20-30% of the phosphorus from a waste containing 10 $mg.l^{-1}$ P while producing a sludge with a phosphorus content of 2-3% by weight. However, Vacker *et al.* (1967) have reported sludge phosphorus contents of 20-22% which would seem to be in excess of metabolic requirements. The percentage of phosphorus removed in the last start-up period was as high as 80% of total $P\text{-}PO_4$ influent from a synthetic wastewater containing 17 mg $P\text{-}PO_4$ $.l^{-1}$. This amount is larger in run 2: a P-removal of 90% in the SBR and 86% for the total system was reached.

The fermentation efficiency of the acidogenic reactor was adequate. Acetate addition for the anaerobic step in the SBR could increase the amount of acidogenic effluent added in the SBR feed, resulting in higher activity of Bio-P bacteria and higher $P\text{-}PO_4$ removal.

Nitrogen removal capacity could be increased by higher NH_4/TKN ratio in the feed. The aerobic condition and this aerobic SBR period could also be extended to keep longer nitrification and hence to lower nitrogen concentrations in the effluent.

CONCLUSIONS

Carbon flux established is appropriate to keep the different microbial populations with effective nutrient removal activity rates. The acidification degree achieved during the current study varied between 28 and 57% of TOC influent concentration. Denitrification occurred in the acidogenic step, which solved the problem of incomplete denitrification by insufficient carbon matter presence in the system. As it is generally

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believed, methanogenic activity began only after denitrification, and greater TOC removal efficiency than each of the single systems (anaerobic digestion or nutrient removal) could be reached.

The system was potentially able to remove phosphorus in all conditions, but P-release and P-uptake rates were unstable, operating conditions merely being the triggering mechanisms. The affinity for a given substrate is not constant for a given organism; it is influenced by both growth rate and ratio of substrate available (Davelar *et al.*, 1978). This could be another possible reason for the instability observed in P-release activity.

In the proposed system, solid loss is low and the existence of zones in which the different populations coexist is possible, because the anaerobic and or aerobic conditions required by the needed populations for C, N and P removal could be assured by the reactor arrangement and operational conditions proposed here. This kind of combined system seems to be able to achieve greater C, N and P removal efficiencies than traditional combined nitrogen and phosphorus removal processes.

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