SEQUENCING BATCH REACTOR AS A POST-TREATMENT OF ANAEROBICALLY TREATED DAIRY EFFLUENT

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Abstract Wastewater from dairy industries, characterized by its high COD content and relative high COD/TKN ratio, requires post-treatment after anaerobic treatment to complete the removal of organic matter and nutrients. Due to its simplicity, robustness and low maintenance costs, Sequencing Batch Reactor (SBR) result an attractive system, specially in the case of small dairy industries in order to comply with the emission standards.

The goal of this work was to determine the operational parameters, optimize the performance, and study the stability of the microbial population of a SBR system for the post treatment of an anaerobic pond effluent. High and stable removal of COD and TKN was achieved in the reactor, which can easily be setup in dairy industries. An active nitrifying population was selected during reactor operation and maintained relatively stable, while the heterotrophic (total and denitrifying) communities were more unstable and susceptible to changes in the operating conditions.

Keywords: post-treatment, desnitrificacion, Sequencing Batch Reactor, aoxic phase….

Introduction

Anaerobic treatment generally needs complementary post-treatment to complete the removal of organic matter as well as nutrients. A subsequent unit is necessary to complete the treatment, according to the emission standards of most part of the countries. Wastewaters from dairy industries are characterized by its high COD content and relative high COD/TKN ratio (Garrido et al., 2001).

In Uruguay, dairy industries are very important and the use of aerobic treatment is a common practice, which is characterized by a relative high energy consumption and biomass production. Despite this, in the last decades, anaerobic treatment systems followed by a post treatment system have shown to be adequate to treat this kind of wastewater. In case of small dairy industries, anaerobic treatment followed by an anoxic/aerobic sequencing batch reactor (SBR) could result attractive due to its simplicity, robustness and low maintenance costs (Arrojo et. al, 2003).

The objectives of this work were to determine the operational parameters, optimize the performance, and study the stability of the microbial population of a SBR system for the post treatment of an anaerobic pond effluent.

Material and Methods

Influent

The effluent from a dairy industrial plant previously treated in a 9400 m^3 anaerobic pond receiving 1300 m^3/d of effluent, was used in the feeding (Table 1). As biodegradable substrate was too low to ensure total denitrification, the SBR was fed with a mixture of 75% effluent from the anaerobic pond and 25 % synthetic raw industrial waste water (prepared with diluted whole milk and NaNO3). In one case, synthetic influent (460 mg/L of Lactose and 380 mg/L of NH4Cl) was used instead of the anaerobic pond effluent.

Table 1. Average characteristic of the effluent from the anaerobic pond and the SBR feed

	Effluent anaerobic pond	Feed SBR
DQO(mg/L)	320	1000
DBO(mg/L)	50	440
TKN (mg/L)	105	110
N-NH4	100	83
pH	7,9	7,5
SST (mg/L)	1500	270
NO_3-N (mg/L)	< 0,5	18
NO_2-N (mg/L)	< 0,2	3,2

Reactor

Cylindrical lab-scale reactor (20 L) has worked continuously for over a year, with two cycles per day and five stages per cycle (Figure 1, Track I and II). After 404 days of working in conditions showed in Track I, some changes in the operation were introduced and the reactor was operated according with Track II. The reactor was fed with 4 L per cycle into 15 L of working volume. Cellular residence time was maintained in 20 day by purging 375 ml of mixed liquor at the end of aerated phase of each cycle. Biomass concentration was between 0,8-2,5 $g.L^{-1}$ of VSS.

Control System

Automation was implemented to supervise and control the operation of the reactor. The system consists of a programmable logic controller (PLC) connected to a computer with a SCADA (Supervisory Control And Data Acquisition) software to provide a totally automatic operation of the reactor. Real-time data acquisition, display and historic trending from on-line sensors (pH, OD, T and ORP) were implemented in the system.

Sampling and analytical methods

SBR was followed by dissolved oxygen, pH and temperature on-line sensors as well as off-line analysis. Total and soluble COD, total and ammonia nitrogen, nitrite and nitrate were determined in the influent, effluent and in the mixed liquor employing techniques in accordance with Standard Methods (APHA, 1995). Volatile and Total Suspended Solids (VSS, TSS) and microbiology techniques were performed in the solids withdraw.

Microbiological methods

In order to study the microbiology of the reactor, three different techniques were applied to the sludge samples.

Nitrifying activity. The rate of oxygen consumption for the nitrifying organisms was measured by respirometric activity tests. The oxygen uptake rate (OUR) for each oxygen consuming group (nitrite oxidizers and ammonia oxidizers) were determined as was described in Cabezas et al., 2004. Substrate concentrations used in the tests were 60ppm N-NH4+, 10ppm N-NO2-. The activities were expressed as mgO2/gSSV.d.

Fluorescence in situ hybridization (FISH) was applied to determine the numbers of nitrifiers (ammonia oxidisers (AOB) and nitrite oxidisers (NOB)) and total Bacteria (from the *Bacteria* and *Archaea* Domain). Hybridization was performed according to Cabezas *et al*, 2004, using CY3 labeled probes Eub338 (targets most *Bacteria*), Arch915 (targets organisms from the *Archaea* Domain), Nso1225 (targets β-proteobacterial AOB including the genus *Nitrosomonas* and *Nitrosospira*), Nit3 (targets *Nitrobacter* spp, NOB), Ntspa662 (targets *Nitrospira* spp, NOB).
T-RFLP techniques were used to evaluate the changes in the microbial composition during reactor operation. For that, the Bacterial population (using the 16S rRNA gene), the denitrifying population (using *nir*S gene) and the ammonia oxidising population (using the *amo*A gene) were studied. 16S rRNA and *nirS* genes T-RFLP were performed according to Braker et al, 2003. For the *amoA* gene T-RFLP, the same conditions described in Horz *et al*., 2000 were used.

Track Studies

Track I. SBR performance was characterized to develop a model, not presented in this paper, by an intensive set of experiences. This study was carried out during 25 cycles within 3 months.

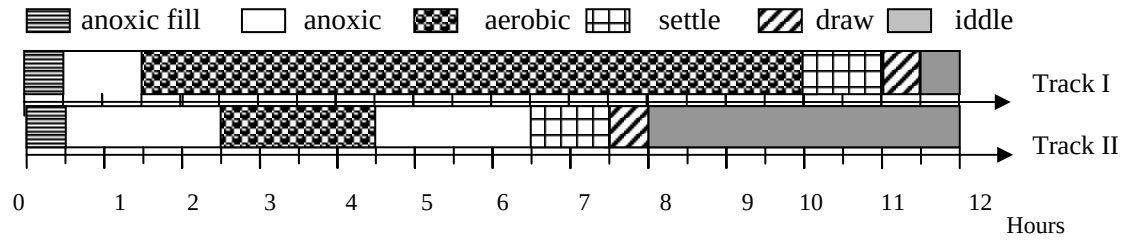

Figure 1. Time table of a cycle in the SBR for Tracks I and II.

*Track II***.** Based on the results obtained, some changes in the operation of the reactor were applied. Aeration phase was shorted from 8,5 to 4 hours. An anoxic phase of 2 hours with instantaneous addition of substrate needed for denitrification (0,5 L of raw dairy effluent) was set at the end of aerobic phase.

Results and discussion

SBR performance

In all working period COD and TKN removal average values were 97 and 98 % with standard deviation of 6,8 and 1,8 respectively, at the end of the cycle. The mean values for respirometric activities were: 350 (ammonia), 130 (nitrite) and 700 (heterotrophs) mgO2/gVSS/d

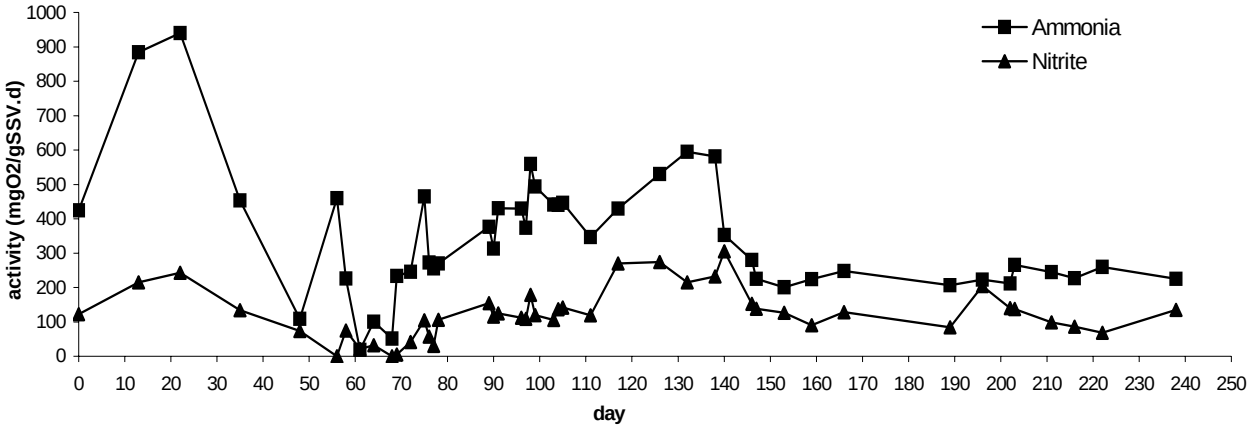

Figure 2. Respirometric activity tests for ammonia oxidising bacteria (ammonia) and for nitrite oxidising bacteria (nitrite)

High variations were detected from the beginning to day 130. More stable but lower activities values were reached after this point. On day 60, all activities dramatically fall; this observation corresponds to a detection of an abnormal input of total and volatile solids in the reactor that comes from the anaerobic pond (probably due to the thermal inversion in autumn). Input of solids from the anaerobic pond should carefully avoid as it seems to be toxic for all organisms in the reactor.

During 216 days of operation organisms hybridising with the AOB and NOB probes were detected in the samples by FISH, indicating that the nitrifying population were maintained inside the reactor (Table 2). The nitrite oxidising population were constituted almost exclusively with bacteria of the genus *Nitrobacter* although organisms form the genus *Nitrospira* were detected by FISH in low amounts in the samples after day 103 (Table 2).

Table 2. Number of organisms detected by FISH from the *Bacteria* domain (probe Eub338), from the β-proteobacterial AOB including the genus *Nitrosomonas* and *Nitrosospira* (Nso1225) from the NOB *Nitrospira* spp (Ntspa662), from the NOB *Nitrobacter* spp. (Nit3).

Reactor operation day	Eub338 (cells/gSSV)	Nso1225 (cells/gSSV)	Nit3 (cells/gSSV)	Ntspa662 (cells/gSSV)
35	4 E+09	2 E+07	1 E+07	N.D.
64	1 E+08	2 E+06	8 E+05	< d.l.
89	9 E+07	3 E+06	2 E+06	< d.l.
103	5 E+08	4 E+06	1 E+06	5 E+03
126	9 E+08	2 E+06	4 E+05	1 E+04
146	3 E+08	3 E+06	1 E+06	9 E+03
216	4 E+08	7 E+06	2 E+06	8 E+04

N.D., not determined <d.l., under the detection limit of the method

Archaea were detected in the biomass after day 35 (data not shown). At this point an increase of the solids in the influent was detected. So, probably methanogenic bacteria (belonging to the *Archaea* Domain) from the anaerobic pond were uptake with the influent.

A low diversity and stable ammonia oxidizing microflora was observed in the reactor

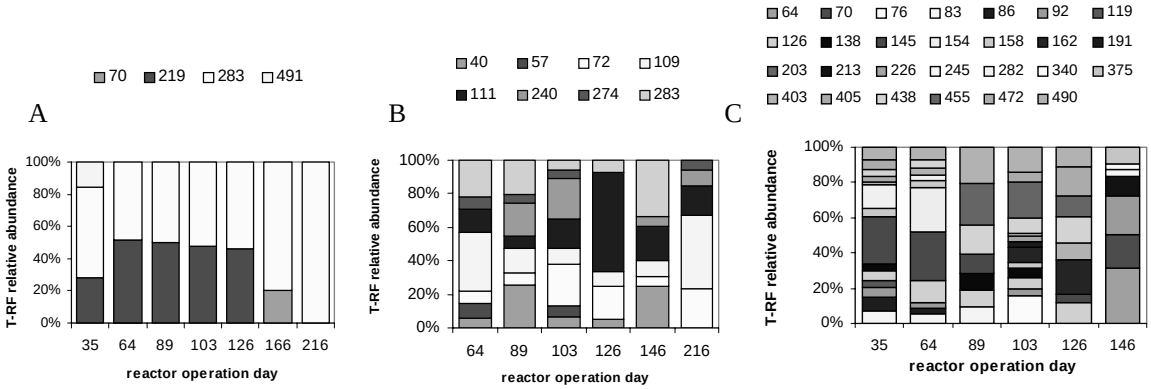

Figure 3. T-RFLP analysis of the sludge samples during time, T-RFLP for *amo*A gene using *Taq*I restriction enzyme (A), T-RFLP for *nir*S gene using the enzyme *Hha*I (B), T-RFLP for the 16S *rRNA* gene using *Msp*I (C).

according to *amo*A T-RFLP (Figure 3.A). Two organisms predominate in most of the samples. According to theoretical calculations from the *amo*A sequences, these TRFs correspond to species from the genera *Nitrosomonas* (219bp) and *Nitrosospira* and *Nitrosovibrio* (283bp). A shift was detected at day 166, TRFs corresponding to species from the genera *Nitrosomonas* were not more detected. At this point the nitrifying activity fall, suggesting that a nitrifying organism with low activity was selected. In the denitrifying community analyzed by the *nir*S T-RFLP, nine peaks were detected and most of them were presented in all the samples (Figure 3.B), the relative proportions of the peaks change during reactor operation. These results indicate the presence of a stable denitrifying community in the reactor but with different denitrifiers predominance along time.

Higher numbers of peaks (27) were detected in the 16S rRNA gene T-RFLP (Figure 3.C). Although some peaks were present in more than one sample, no peak persisted in all the samples. A very different profile was retrieved in the sample taken at day 146, indicating also a shift in the community at this point.

These results indicate that the heterotrophic community presented a high variation during reactor operation. Changes were detected in number of species and in the predominant species.

Track Studies

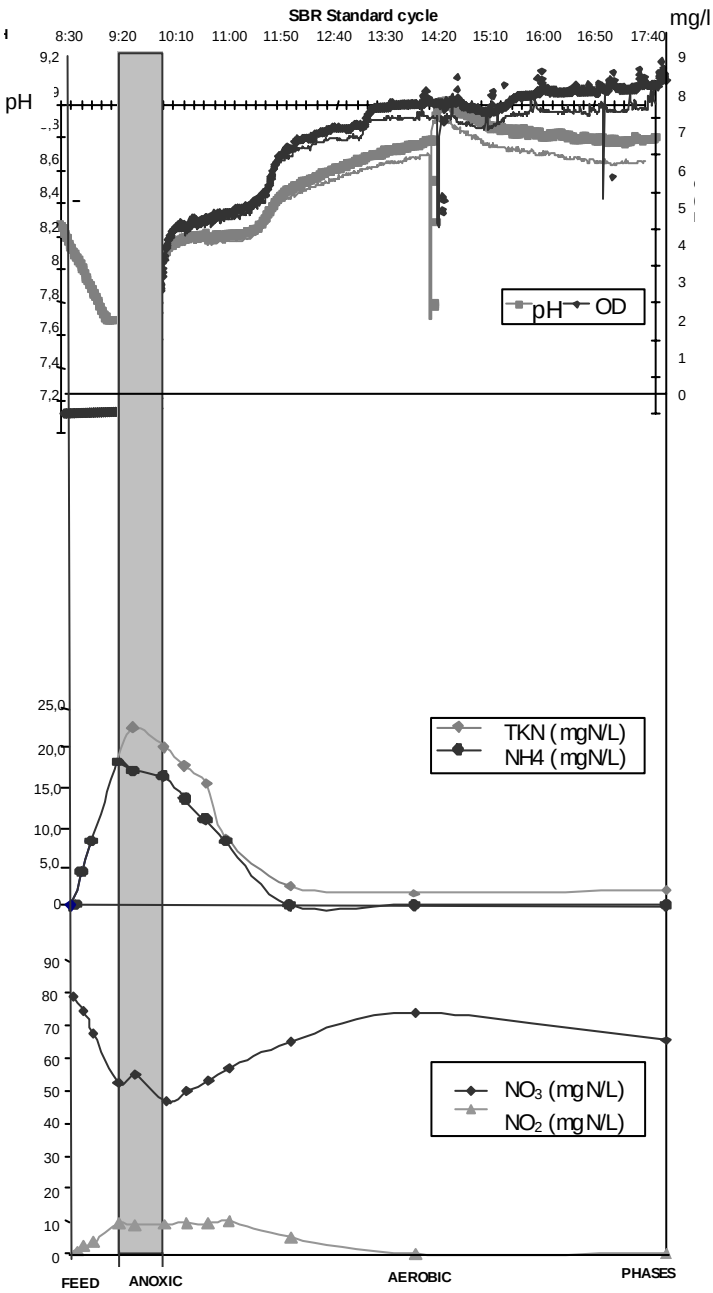

Figure 4. Profiles of: OD, pH, TKN, ammonium and Nitrate and Nitrite for an standard cycle in the SBR - Track I.

Track I. Figure 4 shows standard profiles of on-line sensors and off-line parameters during a cycle. In the anoxic phase pH decreased during feeding, once it was finished pH increased due to denitrification process. At the beginning of aerobic phase, a rapid increase of pH occurred within a short period of time probably due to the stripping of CO_2 out of the system (Chang *et. al*., 1996). OD profile presented two break points due to the disappearance of biodegradable COD and Ammonium and the second one due to disappearance of Nitrite. TKN removal was 97% in the aerobic phase. Denitrification was not complete, even when COD and a high (130 mg/L) concentration of nitrate were present at the end of anoxic phase.

As can be observed for all profiles (Figure 4) after 4 hours of aeration there were no changes in the parameters, concluding that aerated phase duration was excessive.

Track II On base of the results obtained, some changes in the reactor operation were applied to optimize its performance especially with the objective of improve denitrification process. Even reported experience about not inhibitory effect of pH in denitrifying biomass (Dangcong et. al., 2004), optimum pH for denitrification is between 7-8,5 (Orhon, 1998).

The pH measured in the SBR was generally over 8,5 (between 7,9-9,5) and during aerobic phase it generally increased, even when no changes in the rest of parameters were detected. To reduce aeration cost and prevent pH inhibitory effect, the aeration stage was shorted.

To eliminate all Nitrite in the effluent an anoxic phase with addition of biodegradable substrate was set at the end of aerobic phase. For these new conditions, COD and TKN removal were similar as previous cycles, measured pH was lower, varying between 7,5-8,5, and “Nitrate knee” in ORP profile (Figure 5) indicated that denitrification was complete in the first anoxic phase. This result suggests that denitrifying biomass was exposed to an inhibitory effect of pH in the previous operating conditions. For the second anoxic phase, Nitrate value at this point was under detection limits (Figure 6).

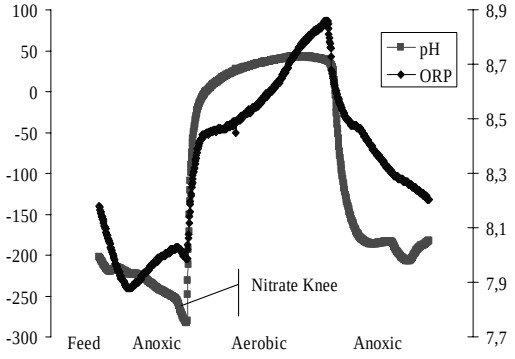

Figure 5 pH and ORP for a cycle Track II

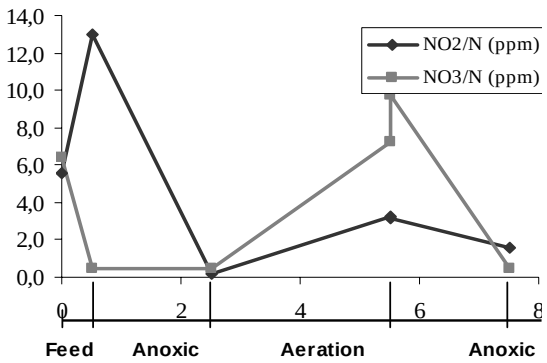

Figure 6 Nitrate and Nitrite for a cycle Track II

Conclusions

According to the microbiological analysis, an active nitrifying population was selected along reactor operation and maintained relatively stable, with the predominance of *Nitrosomonas, Nitrosospira* and *Nitrosovibrio* in the AOB and *Nitrobacter* and *Nitrospira* in the NOB. Operational problems selected a less active but more stable nitrifying population. The heterotrophic (total and denitrifying) communities were more unstable and susceptible to changes in the operation conditions. Despite the good performance obtained, the amount of raw influent added to carry out denitrification can be adjusted to take maximum profit of the anaerobic process.

High and stable removal of COD and TKN were achieved in the reactor that can be easily setup in dairy industries. With the modification described in Track II was possible to eliminate Nitrate. More research is needed to minimize the amount of raw influent added to reduce costs, and to optimize the aerobic and anaerobic stages duration times.

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