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Effect of enzymatic pretreatment and increasing the organic loading rate of lipid-rich wastewater treated in a hybrid UASB reactor

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ABSTRACT

This study aimed at evaluating the effect of increasing organic loading rates and of enzyme pretreatment on the stability and efficiency of a hybrid upflow anaerobic sludge blanket reactor (UASBh) treating dairy effluent. The UASBh was submitted to the following average organic loading rates (OLR) 0.98 $Kg.m^{-3}.d^{-1}$, 4.58 $Kg.m^{-3}.d^{-1}$, 8.89 $Kg.m^{-3}.d^{-1}$ and 15.73 $Kg.m^{-3}.d^{-1}$, and with the higher value, the reactor was fed with effluent with and without an enzymatic pretreatment to hydrolyze fats. The hydraulic detention time was 24 h, and the temperature was 30 ± 2 °C. The reactor was equipped with a superior foam bed and showed good efficiency and stability until an OLR of 8.89 $Kg.m^{-3}.d^{-1}$. The foam bed was efficient for solid retention and residual volatile acid concentration consumption. The enzymatic pretreatment did not contribute to the process stability, propitiating loss in both biomass and system efficiency. Specific methanogenic activity tests indicated the presence of inhibition after the sludge had been submitted to the pretreated effluent. It was concluded that continuous exposure to the hydrolysis products or to the enzyme caused a dramatic drop in the efficiency and stability of the process, and the single exposure of the biomass to this condition did not inhibit methane formation.

1. Introduction

The anaerobic treatment of dairy effluents is feasible and can provide very satisfactory results. Because anaerobic digestion is a necessary tool in the production of clean energy sources, such as hydrogen and methane, biogas production from high-strength wastes will always be of paramount importance for both developed and developing countries [1]. Nevertheless, studies are required to optimize anaerobic reactors that are used to treat fat-rich wastewater for lipids clogging prevention, sludge bed wash-out avoidance and consequently for biogas production. Lipids are slowly degraded due to their low solubility [2], and their presence in the reactors can deteriorate the anaerobic process. The most frequent problems reported in the literature are clogging in fixed film reactors, biomass flotation in reactors with granular beds, the unavailability of the substrate for microorganisms within biofilms, and inhibition of methanogenesis and acetogenesis, which is due to the presence of intermediaries from the degradation of lipids, i.e., long chain fatty acids (LCFA). However, Hwu et al. [3] concluded that in granular anaerobic reactors, sludge bed wash-out is likely to be encountered before the inhibition of methanogenesis. In this way, some alternatives, such as the disposal of filters in the superior part of UASB reactors, have been suggested to minimize the problem of solid expulsion. Strydom et al. [4] evaluated a laboratory-scale hybrid anaerobic digester that combined an upflow sludge blanket and a fixed-bed design for the treatment of dairy effluents with a chemical oxygen demand that ranged from 3.7 $Kg.m^{-3}$ to 10.3 $Kg.m^{-3}$. The efficiency of organic matter removal, expressed as the chemical oxygen demand (COD), was between 90 and 97% when the organic loading rate was between 0.82 and 6.11 kg COD $Kg.m^{-3}.d^{-1}$, respectively. The best results in terms of methane yield were achieved at a hydraulic retention time (HRT) of 1.9 d, while the data also showed that the maximum operational potential of the digester had been reached because the methane yield dropped with a lower hydraulic retention time. Cordoba et al. [5] compared two anaerobic systems of diluted dairy wastewater treatment: a biological filter with polyurethane foam as a support and a UASBh with its gas/liquid/solid separator substituted by a polyurethane foam stream bed. With organic loading rates from 1 to 8 $Kg.m^{-3}.d^{-1}$, the hybrid configuration showed better results, which reached around 90% COD removal at the highest load. Najafpour et al. [6] proposed a hybrid tubular configuration that was called a UASFF (hybrid reactor with an upflow fixed film part over an UASB) to

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Abbreviations and notations

AB	anaerobic biodegradability.
BA	bicarbonate alkalinity (g CaCO3.m^{-3}).
COD	chemical oxygen demand (g.m^{-3})
COD_eff	effluent COD (g.m^{-3})
COD_inf	influent COD (g.m^{-3})
COD_F	filtered chemical oxygen demand (g.m^{-3}).
COD_T	total chemical oxygen demand (g.m^{-3}).
D	diameter.
HAc	acetic acid
k_1^{app}	apparent first order kinetic constant (h^{-1}).
K_{CH4}	kinetic constant of methane production (h).
L	length.
LCFA	Long chain fatty acids.
M_{CH4}	total amount of methane production
M_{COD}	mass of organic matter provided in the AB tests
OC	Operational condition.
OLR	organic loading rate (kg COD.m^{-3}.d^{-1}).
P_{CH4}	methane production.
$P_{CH4máx}$	accumulated methanogenic production.
R^2	determination coefficient.
r_i	initial rate of substrate consumption (g.m^{-3}.h^{-1}).
S_0	initial substrate concentration (g.m^{-3}).
SEM	scanning electronic microscopy.
SMA	specific methanogenic activity (mol of CH_4.gSTV^{-1}.h^{-1})
SMP	specific methanogenic production (cm^3CH_4.mgCOD^{-1}).
Sr	residual substrate concentration (g.m^{-3}).
SS	suspended solids
TVA	total volatile acids (gHAc.m^{-3}).
VSS	volatile suspended solids
U	enzymatic unity.
UASBh	hybrid upflow anaerobic sludge blanket.
UASFF	Hybrid reactor with an upflow fixed film part over a UASB.

decrease the hydraulic retention time and the start-up period for the oil mill effluent treatment. High chemical oxygen demand removals of 89 and 97% at a HRT of 1.5 and 3 days were achieved, respectively, while at the highest organic loading rate, a methane yield of 0.346 m^3 CH_4/kg COD removed was obtained. As another alternative for the improvement of the rate of lipid degradation and the reduction of the problems that related to its presence, hydrolysis pretreatment has been proposed by many authors. According to Mendes et al. [7], the use of lipases reduces the suspended solid and lipid levels and enables better readiness in the anaerobic treatment by minimizing the formation of oil films in tubing. Lipases act in the organic-aqueous interface as catalysts in hydrolysis reactions of carboxylic-ester linkages, which release fatty acids and glycerol, which has anaerobic biodegradability around 100% and methane yield coefficient of 0.306 m^3 CH_4/kg acidified glycerol, according to López et al. [8]. By using an enzymatic pool obtained by *P. restrictum* solid state fermentation, Leal et al. [9] observed that the previous enzymatic treatment enhanced the global efficiency of a pilot-scale UASB reactor treating dairy wastewater and also increased the initial degradation rate of the organic matter. Thus, to combine the advantages of the enzymatic pretreatment and the hybrid configuration, this study aimed at evaluating the effect of increasing organic loading rates and of enzyme pretreatment on the stability and efficiency of a hybrid upflow anaerobic sludge blanket reactor (UASBh) treating dairy effluent. For both, UASB reactor was fed with real dairy effluent submitted to increasing loads of feeding. Moreover, with the higher organic load, enzymatic pretreatment was carried through to evaluate its effect on the anaerobic process. Specific methanogenic activity and anaerobic biodegradability tests were also conducted to evaluate the methanogenic potential and to allow apparent kinetic parameters inference.

2. Materials and methods

2.1. Experimental design

The system was submitted to organic load rates (OLR) of 0.98 Kg.m^{-3}.d^{-1}, 4.58 Kg.m^{-3}.d^{-1}, 8.89 Kg.m^{-3}.d^{-1}, 15.73 Kg.m^{-3}.d^{-1} and 14.82 Kg. m^{-3}.d^{-1} (named operational conditions (OC) 1, 2, 3, 4 and 5, respectively) with raw and pretreated wastewater. The operation time and the average influent and effluent COD in all of the operational conditions are presented in Table 1. The influent COD concentrations in OC1, OC2 and OC3 were obtained by diluting the dairy wastewater. In OC4, the reactor was fed with non-diluted wastewater, while in OC 5, the reactor was fed with non-diluted pre-hydrolyzed wastewater. The hydrolysis reaction was performed with porcine pancretin. The reactor was monitored for 183 days and at the end of each OC, samplings throughout the height of the reactor were taken to obtain the concentration profiles of the substrates (COD, lipids, carbohydrates and protein) and metabolic products (volatile acids and bicarbonate alkalinity). At the beginning of each OC, the reactor was filled with 3.3×10^3 cm^3 of previously adapted granular sludge. During each OC, the loss of solids and the increase of the sludge blanket in the reactor were quantified. The sludge production was determined by measuring the sludge bed volume and determining its solids content. The foam bed was also dismounted at the end of each operational condition to measure the volume of entrapped sludge. The sludge losses were measured in a sieve (0.5 mm pore diameter) that was placed in the reactor's output. At the beginning and end of the experiment, the sludge's specific methanogenic activity (SMA) was determined. The content of methane was measured by liquid displacement. To evaluate the methanogenic potential and the occurrence of some kind of inhibition due to the rising of organic loading rate applied in the

Table 1
Operation time, average* influent and effluent COD and average* removal efficiency along the adaptation period and under all operational conditions.

	Operation time	COD_Finf (g.m^{-3})	COD_Tinf (g.m^{-3})	COD_Feff (g.m^{-3})	COD_Teff (g.m^{-3})	RE (COD_F -%)	RE (COD_T -%)
Adaptation	30	556	610	37	68	89	93
OC1	30	1082$^{\mathbf{C}}$	1014$^{\mathbf{C}}$	104$^{\mathbf{C}}$	143$^{\mathbf{C}}$	90$^{\mathbf{A}}$	85$^{\mathbf{A}}$
OC2	25	3905$^{\mathbf{B, C}}$	4087$^{\mathbf{C}}$	427$^{\mathbf{B, C}}$	665$^{\mathbf{B, C}}$	89$^{\mathbf{A}}$	83$^{\mathbf{A}}$
OC3	46	7219$^{\mathbf{A, B}}$	8515$^{\mathbf{B}}$	346$^{\mathbf{B, C}}$	576$^{\mathbf{B, C}}$	95$^{\mathbf{A}}$	93$^{\mathbf{A}}$
OC4	40	10468$^{\mathbf{A}}$	13499$^{\mathbf{A}}$	2271$^{\mathbf{A, B}}$	2592$^{\mathbf{B}}$	76$^{\mathbf{A}}$	79$^{\mathbf{A}}$
OC5	46	6063$^{\mathbf{B}}$	9930$^{\mathbf{A, B}}$	3937$^{\mathbf{A}}$	5115$^{\mathbf{A}}$	30$^{\mathbf{B}}$	46$^{\mathbf{B}}$

COD_inf — influent COD; COD_eff — effluent COD; RE — average removal efficiency.
Different letters within columns denote significant difference ($p<0.01$) by *t* Test.
* The averages were calculated using the last four values obtained in each OC to eliminate the variation due to system adaptation.