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Reduction of scum accumulation through the addition of low-cost enzymatic extract in the feeding of high-rate anaerobic reactor

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ABSTRACT

This work evaluates the reduction of scum accumulation on the top surface of upflow anaerobic sludge blanket (UASB) reactors by the addition of hydrolytic enzymes in their feed. For over 1 year, two UASB reactors of 1.4 L were maintained at 30 °C and continuously fed with synthetic domestic wastewater (containing 150 mg/L of soybean oil) under a hydraulic retention time of 10 h.

The Control reactor was only fed with synthetic wastewater. Beginning at the 226th day of operation, low-cost hydrolytic enzymes (obtained by solid-state fermentation of *Aspergillus terreus*, a fungus isolated from a primary sewage sludge) were added into the feed of the other reactor (Test) for a lipase activity of 24 U/L, considerably reducing the formation of scum. In the Test reactor, the scum showed oil and grease (O&G) concentration between 0.8 and 1.3 g/L and an accumulation rate of 20 to 27 mg O&G/d. In the Control reactor, the scum had values twice as high (1.5–2.5 g/L and 34–51 mg O&G/d, respectively) and there were more operational problems. During the entire period of operation, both reactors presented high chemical oxygen demand removal (>80%), with no loss of effluent quality due to the addition of the enzymes.

Key words | enzyme, fat, scum, sewage treatment, solid-state fermentation, upflow anaerobic sludge blanket reactor

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INTRODUCTION

In an upflow anaerobic sludge blanket (UASB) reactor, a diverse microbial population converts biodegradable organic compounds into by-products in solid (biological sludge), liquid (water and mineral salts – treated effluent) or gaseous (biogas) forms. In this reactor, the sludge retention is based on the formation of well settleable sludge aggregates (flocs or granules), and on the application of an internal gas–liquid–solid separation (GLSS) device (Chernicharo *et al.* 2015). The retention of high biomass concentration (up to 80 g/L) permits operation under high volumetric loading rates and low hydraulic retention times, plus it very efficiently removes organic matter from industrial wastewaters (van Lier *et al.* 2015). The UASB reactor is also applied for domestic wastewater treatment and the technology is widely employed in warm climate regions, such as Brazil and other Latin American countries (Chernicharo *et al.* 2015).

However, when UASB reactors are used to treat wastewater that contains fat and other low-density materials, scum forms, and a layer of floating materials accumulates on the liquid surface of the settling compartment and in the internal part of the GLSS device (Souza *et al.* 2006; Pereira *et al.* 2009). Scum accumulation impairs the performance of UASB reactors because it blocks the flow and the regular recovery of biogas, imposes loss of dissolved biogas into the effluent and contributes to the continuous release of organic content through the treated effluent. Several mechanical methods for scum removal have been studied (Pereira *et al.* 2009; Chernicharo *et al.* 2015), but to the best of our knowledge, no previous study has reported the application of low-cost enzymes produced by solid-state fermentation (SSF) to solve the problem of scum in anaerobic reactors.

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The use of lipases in aerobic and anaerobic reactors to treat industrial wastewaters with high fat content has been evaluated, due to the effective action of enzymes on the hydrolysis of oil and grease (O&G), for the prevention of operational problems. However, the application of enzymes in wastewater treatment must be economically viable. An SSF process can be applied to produce low-cost enzymes, using agro-industrial waste as substrate (Aguieiras *et al.* 2018).

Enzymes can be applied as two different products: a solid enzymatic preparation (SEP), as a product of the SSF process, and as a liquid solution extracted from the same SEP. To reduce O&G accumulation on anaerobic reactors treating industrial wastewater, Ferreira-Leitão *et al.* (2017) discuss results obtained from the application of enzymes produced by SSF in a pre-hydrolysis step as well as directly in the reactor itself. The application of lipases in domestic sewage or its scum is still poorly explored. Subsequent studies of our group found that pre-hydrolysis of scum from a UASB reactor treating domestic wastewater, based on SEP of *Aspergillus terreus* and further anaerobic degradation, can enhance methane production (Lima *et al.* 2018). The enzymatic extract produced from SEP can be applied to wastewater treatment in the inflow or recirculation lines of the reactor or directly at its top (where scum is mostly accumulated), according to operational circumstances, reactor characteristics, and expected results. These strategies allow the elimination of a hydrolysis step and favor continuous operation. Therefore, the general objective of this study is to evaluate the effect of the application of enzymatic extract containing lipase on scum accumulation of UASB bench reactors treating domestic wastewater.

METHODS

Synthetic sewage used in reactor feed

The synthetic sewage used to feed the bench reactors was adapted from Torres *et al.* (1996), with the addition of oil to increase its concentration and to facilitate and accelerate the formation of the suspended layer and the accumulation of scum on the top of the reactors as in real-scale units. The medium contained (in mg/L) sucrose 56, starch 182.4, cellulose 54.4, meat extract 332.8, soybean oil 150 (emulsified by addition of commercial detergent drops), NaCl 250, $MgCl_2.6H_2O$ 7, $CaCl_2.2H_2O$ 4.5, and $NaHCO_3$ 320.

Obtaining the enzyme extract

The SEP was produced using SSF with the fungus *Aspergillus terreus* and an 80:20 (% w/w) mixture of babassu cake (a residue from the pressing of almond for the extraction of babassu oil) and primary sewage sludge (dried mixture of primary sewage sludge and scum) as described by Lima *et al.* (2018). The babassu cake was ground and sieved to separate the fraction $\leq$1.18 mm for fermentation. The primary sludge was screened to separate interfering materials (e.g. plastics and wood) and sieved to separate the fraction $\leq$1.18 mm for fermentation.

The fermentation was conducted in 600 mL beakers containing 15 g of the residue mixture at 30 °C, with moist air circulation and 65% initial moisture, corrected with the spore suspension for an initial concentration of 10^7 spores/g. After 48 h, the fermented residue mixture was dried for 48 h in an oven at 40 °C, with dry air injection, obtaining a SEP with lipase activity of 10 ± 3 U/g, and humidity <10%, and was stored at −20 °C.

The extraction of the enzymes from the SEP was adopted to avoid interference of residue mixture solids in UASB reactors' operation. At the end of the fermentation, 5 mL of sodium phosphate buffer (50 mM, pH 7.0) per gram of SEP was added. Extraction of the enzymes was performed in a shaker at 30 °C and 200 rpm for 20 min. Subsequently, the blend was manually pressed to obtain crude enzyme extract.

The lipase activity was determined by titration method using olive oil as a substrate (Freire *et al.* 1997). One unit of lipase activity has been defined as the amount of enzyme that releases 1 μmol of free fatty acid per minute under the assay conditions.

Operation of UASB bench reactors

The study was conducted based on two UASB bench reactors with a useful volume of 1.4 L each, both installed in a room with controlled temperature of 30 ± 1 °C. The reactors were inoculated with sewage sludge from a UASB reactor under operation at the Experimental Center of Environmental Sanitation of the Federal University of Rio de Janeiro, Brazil, presenting 29.3 g/L of volatile suspended solids. The reactors were continuously fed with synthetic domestic wastewater and maintained under a hydraulic retention time of 10 h.

The study was divided into two phases of operation that lasted 226 and 132 days, counting only the days of continuous feeding, without interruptions. In the first phase (226 days), both reactors were fed with synthetic sewage, to observe the accumulation of scum on the liquid surface and related operational problems. In the second phase (132 days), to evaluate the effect of lipase on fat accumulation and performance of the UASB process, the enzyme extract was added in the inflow line of the Test reactor, according to the volume required for a lipase activity of 24 U/L. This value was based on the ratio of 240 U/L to 1,500 mg/L of O&G employed in a previous study (Lima *et al.* 2018). During this phase, the Control reactor continued to be fed with synthetic domestic wastewater, but without the addition of the enzyme extract.

At the end of the first phase (day 226) and on days 69 and 132 of the second phase, the accumulated scum was removed from the top of the reactors and quantified by O&G analysis. The GLSS device and pipes were cleaned and the continuous feed resumed. To observe the formation of the scum layer over time and to remove the samples for O&G analysis, the reactors operated without sealing the top cover, which precluded monitoring of biogas production. Thus, the monitoring of both reactors was done by pH, chemical oxygen demand (COD), total alkalinity (TA), and volatile fatty acids (VFA) analyses. VFA and TA were determined according to Dilallo & Albertson (1961) and Ripley *et al.* (1986), respectively. Other parameters were measured according to *Standard Methods for the Examination of Water and Wastewater* (APHA/AWWA/WEF 2005).

RESULTS AND DISCUSSION

During the first phase of operation (226 days), without addition of enzyme extract, scum accumulated at the top of both reactors. After 226 days of continuous operation, the material accumulated on the top of one of the reactors achieved up to 3.3 g O&G/L (Figure 1), proving that this accumulation occurs even though there were no particulates (e.g. food leftovers, fruit and vegetable skins, hair, paper, cotton, cigarette butts, plastics, and sand particles) and other low-density materials (e.g. fats, waxes, and soaps), which facilitate the formation of scum (Souza *et al.* 2006). In this phase, both reactors often suffered inflow line clogging, outflow obstruction, and consequently overflow.

During the second phase and with the application of enzyme extract in the inflow line of the Test reactor, the continuous feed operation of both reactors was interrupted at the 69th and 132nd day of operation for surface layer removal and O&G measurement. The O&G concentrations in the material removed from the top of both reactors are shown in Figure 1. In the two samplings, the values for the Control reactor were 1.8 times higher (2.5 and 1.5 g/L) than for the Test reactor (1.3 and 0.9 g/L), due the addition of the enzyme extract in the Test reactor. The same behavior occurred for the scum accumulation rates: 50.6 and 33.9 mg/d in the Control reactor and 26.8 and 19.5 mg/d in the Test reactor (Figure 1). During 2 months of continuous feed of synthetic sewage, scum formed two times faster in the Control reactor than in the Test reactor.

In both reactors, O&G concentration and scum accumulation rate were lower during the second sampling period of the second phase (from 69th to 132nd day). Although continuous feeding periods of both reactors were similar in the second phase (69 days in the first period and 63 days in the second), the reactors remained much

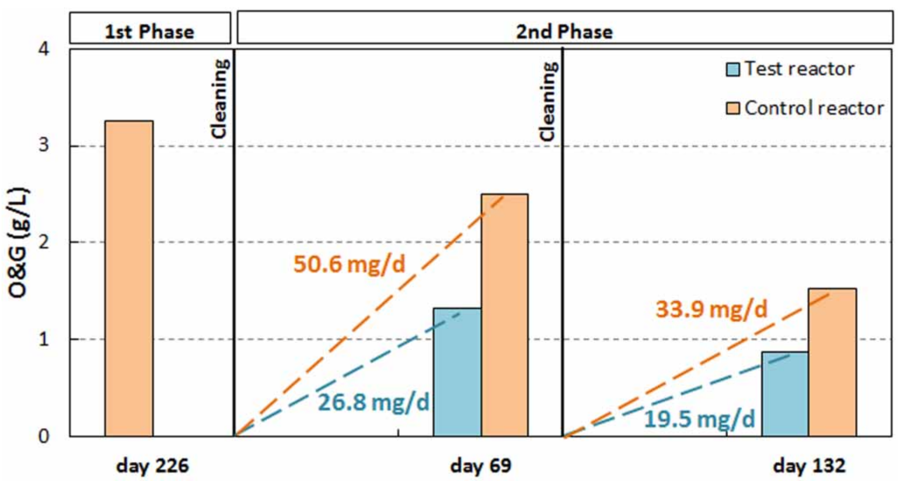

Figure 1 | O&G concentration (g/L, y-axis) in the scum removed from the reactors at 226th day of operation of the first phase (Control reactor) and at 69th and 132nd day of operation of the second phase (Control and Test reactors) and scum formation rate (mg O&G/d) at 69th and 132nd day of operation of the second phase (Control and Test reactors).

longer in batch during the second period, without feeding (due to some events of lack of energy), allowing some degradation of accumulated material.

Figure 2(a) illustrates the accumulated scum layer on the GLSS device after 226 days of continuous operation (first phase) of the Control reactor prior to its removal for O&G analysis. Figure 2(c) shows lower accumulation of scum in the Control reactor during the second phase (63 days) when compared to the performance of the same reactor during the first phase (Figure 2(a)). It may be justified due to the shorter period of continuous operation (63 days). Even so, it is possible to see the blocking of the gas outlet in the GLSS device and a fat plate on the Control reactor surface. A very different image is observed for the Test reactor: both the GLSS device and the reactor surface were clean, with no apparent accumulation of scum (Figure 2(b)).

Thus, the O&G concentration results prove that the proposed goal was achieved. The addition of enzyme extracts rich in lipases from a strain of *A. terreus* isolated from primary sewage sludge helps to reduce scum accumulation in UASB reactors treating domestic wastewater. However, the effect of enzyme addition on UASB performance and effluent quality should be considered: enzyme must be effective on fat hydrolysis and not affect activity of microorganisms. Table 1 presents operational results from both operational phases and proves that, besides reducing scum accumulation, the addition of enzyme did not significantly influence the performance of the Test reactor.

Figure 2 | Aspect of the UASB reactors' top in different phases of operation. (a) Scum deposited on the GLSS device of one of the reactors after 226 days of feeding without addition of enzyme (first phase). (b) GLSS device and surface of the Test reactor (with addition of enzyme in the feed) at 69th day of operation (second phase). (c) Internal part of the GLSS device and surface of the Control reactor (without addition of enzyme in the feed) at 132nd day of operation (second phase).

Table 1 | Summary of the results obtained during the operation of the Control and Test reactors (30 °C, HRT 10 h)

	1st phase – 226 d without EE		2nd phase – 132 d	
Parameter	**Test**	**Control**	**Test with EE**	**Control**
pH influent	7.7 ± 0.4	7.7 ± 0.4	7.6 ± 0.3	7.8 ± 0.4
pH effluent	7.8 ± 0.3	7.9 ± 0.3	8.0 ± 0.2	8.1 ± 0.2
COD influent (mg/L)	724 ± 177	724 ± 177	696 ± 196	660 ± 189
COD effluent (mg/L)	76 ± 47	75 ± 44	80 ± 37	63 ± 25
COD removal (%)	89.1 ± 7.2	89.1 ± 7.4	88.7 ± 3.5	90.9 ± 2.9
VFA influent (mg/L)	38 ± 10	38 ± 10	72 ± 21	52 ± 18
VFA effluent (mg/L)	16 ± 4	19 ± 7	32 ± 12	24 ± 10
TA influent (mg/L)	182 ± 43	182 ± 43	219 ± 49	200 ± 51
TA effluent (mg/L)	258 ± 64	277 ± 64	329 ± 32	314 ± 36
VFA/TA effluent	0.07 ± 0.04	0.07 ± 0.03	0.10 ± 0.03	0.08 ± 0.05
O&G scum (mg/L) – 1st sample (69 days after cleaning)			1,321 ± 371	2,496 ± 738
O&G scum (mg/L) – 2nd sample (63 days after cleaning)			877 ± 429	1,527 ± 92
Scum formation rate (mg O&G/d) – 1st sample			26.8 ± 7.5	50.6 ± 15.0
Scum formation rate (mg O&G/d) – 2nd sample			19.5 ± 9.5	33.9 ± 2.0
Average scum formation rate (mg O&G/d)			23.1 ± 5.2	42.3 ± 11.8

EE, with addition of enzyme extract for a lipase activity of 24 U/L; COD, chemical oxygen demand; VFA, volatile fatty acids; TA, total alkalinity; O&G, oil and grease.

The products derived from fat hydrolysis through the use of lipases are glycerol and long-chain fatty acids (LCFA). The former has no inhibitory effect on anaerobic microorganisms (Nakazawa *et al.* 2015). However, the latter are known inhibitors of methanogenic archaea (Pascale *et al.* 2018). The degradation of LCFA and glycerol by the anaerobic biomass increased the formation of VFA, whose toxicity is related to the non-ionized form of these acids. The non-ionized form, which increases in acidic pH, is much more toxic (Vidal *et al.* 2000).

Therefore, the pH of the reaction medium is important not only for the stability of enzymatic activity (lipases from *A. terreus* are more stable at pH 6 to 9 (Sethi *et al.* 2016)) but also to minimize inhibitory effects. The values in Table 1 indicate that the pH did not change as a result of the addition of enzymatic extract in the feed, because the values are similar for both reactors during the operational period. During the second phase of operation, the pH inside the Test reactor (effluent pH) remained around 8.0 and adequate for enzyme stability and activity of methanogenic microorganisms (Chernicharo 2007).

Due to its composition, the enzyme extract increased TA by 20% and the VFA by almost 90% in the feed of the reactor (Table 1). However, acidity increase was balanced by microbial activity, which uses VFA as a substrate (56% reduction in VFA) and releases metabolites, such as ammonia from protein degradation, increasing TA by almost 50%. This equilibrium is demonstrated by the 43% increase of the VFA/TA ratio from the first to the second phase of the Test reactor, which still remains according to acceptable values for the anaerobic digestion process (Chernicharo 2007).

Analyses of influent and effluent COD of both reactors (Figure 3, Table 1) were used to evaluate the organic matter removal efficiency and performance of the UASB reactors. COD removal efficiency was always above 80%, indicating good performance during both phases of operation. During the second phase, the addition of enzyme in the inflow line of the Test reactor did not significantly change the input COD (the increase of 5% in relation to the Control reactor is within the measurement error of the analysis method) but increased the effluent COD by 27%. However, effluent COD remained within effluent discharge guidelines. Therefore, it can be stated that the enzyme extract does not affect performance of the UASB reactor and the effluent quality.

Having proven the effectiveness of the application of enzymatic extract to reduce scum in bench reactors, a technical and economic feasibility analysis of this application on an industrial scale is necessary. The growth and expansion of the global market for enzymes proves the feasibility of application on an industrial scale. The global enzymes market, including lipases, faces an important growth due

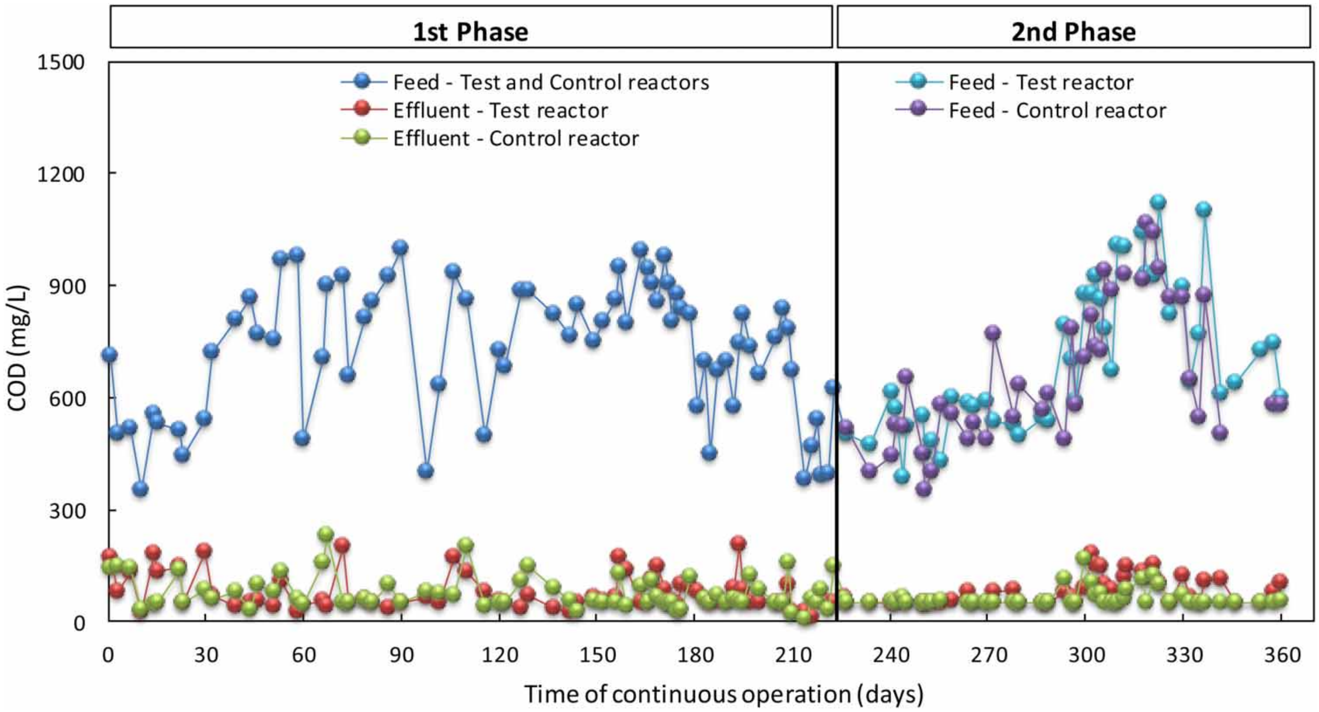

Figure 3 | COD values in the feed and treated effluent of the Control and Test reactors throughout the operational period.

to the upsurge in demand. It is projected to reach US$10,519 million in 2024, registering a growth rate of 5.7% from 2018 to 2024. The boost of the global enzymes market is greatly driven by high demand for effective enzyme-based pharmaceuticals, but renewable energy sources such as biofuels also contribute for it (Srivastava & Srivastava 2018).

Handling and safety issues of enzymes and their high sensitivity to temperature and pH hamper the market growth. Conversely, awareness about the application in protein engineering technology and high market potential in the untapped emerging economies are expected to provide lucrative growth opportunities for the market expansion. North America dominates the enzymes market and US manufacturers are focusing on the expansion of the production capacity due to growing industrial demands, including new technologies to cater for the contemporary needs of the bioenergy industry (Srivastava & Srivastava 2018).

Previous studies proved that the SSF is very attractive from an economic point of view. Castilho *et al.* (2000) made an economic analysis of the production of 100 m^3 per year of a *Penicillium restrictum* lipase concentrate for processes based on submerged and solid-state fermentations. The SSF process provided unitary product costs 47% lower than the lipase market price, payback time of 1.5 years, return on investment of 68% and internal return rate of 62% for a 5-year project life. Damasceno *et al.* (2018) performed a preliminary techno-economic analysis of the application of a mixture of biosurfactant and crude enzyme consortia, produced by SSF with *Penicillium brevicompactum*, to treat 234,000 m^3 per year of poultry slaughterhouse wastewater. The economic analysis revealed that this alternative technology has installation and operating costs about 60% and 6% lower than the conventional plant (using floaters/chemicals). In addition, different production methods have been developed in order to reduce costs and make feasible applications in lower capital sectors such as waste treatment (Aguieiras *et al.* 2018; Ávila *et al.* 2019).

CONCLUSIONS

The addition of enzymes extracted from SEP produced by SSF of fungus *A. terreus* in the inflow line of a high-rate anaerobic reactor (UASB) treating synthetic domestic wastewater obtained satisfactory COD removal results, and differences in the performance of reactors with (Test) and without addition of enzymes (Control) were not observed. However, the rate of scum accumulation (measured as

O&G) was 1.8 times less in the Test reactor, evidencing the action of lipases on the degradation of fat present in raw influent sewage. Therefore, the addition of enzyme extracts rich in lipases contributed to the reduction of scum in UASB reactors treating domestic wastewater and did not affect the quality of the treated effluent.

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