Continuous Enzymatic Prehydrolysis Treatment of High-Fat Wastewater

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Summary

A lipolytic fermented solid was produced by solid-state fermentation of *Rhizopus microsporus* CPQBA 312-07 DRM on a mixture of sugarcane bagasse and sunflower seed meal and used, in a packed-bed bioreactor, to pretreat a high-fat wastewater from a meat and sausage processing factory located in São José dos Pinhais, State of Paraná, Brazil. With a hydraulic residence time of 24 h, this pretreatment not only reduced the wastewater's oil and grease content by up to 96 %, but also increased its 5-day biochemical oxygen demand to chemical oxygen demand (BOD_5/COD) ratio. This ratio was only 0.19 in the raw wastewater, indicating poor biodegradability, but increased to 0.55 in the pretreated wastewater, indicating that it had a sufficiently high biodegradability to be sent to a traditional anaerobic digestion or activated sludge process. After 96 days of operation of the packed bed, a microbiological analysis showed that *R. microsporus* was still present and viable in the fermented solid. Our work shows that a continuous packed-bed bioreactor containing fermented solid produced by *R. microsporus* has good potential for the treatment of high-fat wastewater.

Key words: enzymatic hydrolysis, lipases, *Rhizopus microsporus*, high-fat wastewater, packed--bed bioreactor

Introduction

Wastewaters with a high oil and grease (OG) content (above 150 mg/L), such as those produced by dairy and meat processing industries, are extremely difficult to degrade biologically. Aerobic treatment in aerated lagoons or activated sludge reactors is especially problematic since the adsorption of lipids onto the cell wall hinders the diffusion of O_2 into the microbial cell, thereby slowing its metabolism. This may lead to the formation of a filamentous sludge and reduce the overall removal of organic matter (*1–3*).

High-fat wastewaters can be treated with lipases (triacylglycerol hydrolases, E.C.3.1.1.3) to hydrolyze the fats to glycerol and free fatty acids. This improves the biodegradability of the wastewater, as indicated by an increase in the ratio between the biological oxygen demand and the chemical oxygen demand (*i.e.* the BOD_5/COD ratio). However, even though excellent OG removal can be achieved, it is too costly to use either commercial

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lipases (*4,5*) or lipases produced in traditional submerged fermentation processes (*6*). One strategy to increase the competitiveness of this technology is to produce the lipases by solid-state fermentation (SSF), since production costs are predicted to be only 30 % of those in submerged fermentation, especially if solid agro-industry residues are used as substrates (*7*). An advantage of the SSF process is that it is not necessary to recover and concentrate the lipases at the end of the fermentation. Rather, the fermented solids containing the lipolytic activity, which will hereafter be referred to as lipolytic fermented solids, can be added directly to the effluent.

Most previous studies of the use of lipolytic fermented solids to pretreat high-fat effluents have been undertaken in small batch reactors (containing 70 to 400 mL of effluent) with 0.1 to 5.0 % (*m/V*) of fermented solid (*8–13*). The addition of 0.1 % (*m/V*) of fermented solid to a small-scale continuous-flow activated sludge reactor has also been studied, in order to establish the influence of adding the enzymatic preparation directly in the biological treatment (*14*).

Packed-bed bioreactors allow the possibility not only of obtaining higher solids to wastewater ratios than those that have been studied to date, but also of having a continuous process during which the same batch of fermented substrate can be used for long periods to pretreat large volumes of wastewater. However, their use for this application has not yet been investigated. The objective of the present study is therefore to investigate the effectiveness of a continuous process, carried out in a packed-bed bioreactor filled with a lipolytic fermented solid, for the enzymatic prehydrolysis of a high-fat wastewater produced by a meat and sausage processing factory. The lipolytic fermented solid was produced using *Rhizopus microsporus* CPQBA 312-07 DRM, since it has proved effective in previous studies carried out in batch reactors (*13*). The pretreatment was evaluated based on the removal of OG and the degree of increase in the BOD_5/COD ratio. Our laboratory-scale study shows that the packed-bed bioreactor does indeed have potential and therefore lays the basis for further studies of the scale-up of the enzymatic pretreatment process.

Materials and Methods

Fungal strain and media

Rhizopus microsporus CPQBA 312-07 DRM was grown on potato dextrose agar (PDA) at 30 °C for 5 to 7 days. Spores were harvested in a previously sterilized Tween 80 solution (0.01 %, by volume). The spore concentration in the suspension was determined using a Neubauer chamber.

Production of the lipolytic fermented solid

The solid with lipolytic activity was produced by SSF, using a mixture of sugarcane bagasse and sunflower seeds (3:1 by dry mass basis). The sugarcane bagasse (donated by Usina de Álcool Melhoramentos, Jussara, Paraná, Brazil) was washed 3 times with tap water, then dried at 100 °C for 24 h. The sunflower seed (purchased at a local market) was triturated and screened to obtain particles between 0.85 and 1.4 mm. A mass of 40 g of the mixture was transferred into a 1000-mL Erlenmeyer flask and sterilized at 121 °C for 15 min.

A 10-mL aliquot of a *R. microsporus* spore suspension, corresponding to $1.2 \cdot 10^9$ spores, was transferred to 160 mL of a previously sterilized phosphate buffer solution (0.1 M, pH=7.0). This was then used to inoculate the Erlenmeyer flask containing the sterilized solid substrate. The flask was agitated vigorously manually, and then incubated at 40 °C for 18 h. After incubation, a 5-gram sample was taken for lipase activity measurement. Then the solid was transferred to a plastic bag and stored at –18 °C in a freezer. This procedure was repeated until 2 kg of lipolytic fermented solid were obtained.

Collection and characterization of the wastewater

The high-fat wastewater was donated by a meat and sausage processing factory in São José dos Pinhais, State of Paraná, Brazil. This industry does not have a slaughterhouse; it simply processes meat for the production of bacon and sausages. The collection was made after the static screening unit in a 250-litre plastic barrel. After homogenization, the pH, COD, BOD_5 and OG of the raw wastewater were determined. It was then stored at –18 °C, in various smaller recipeints. Two collections were made, on March 25 and on May 27 of 2010. The wastewater had a high organic matter content and an OG content of around 600 mg/L (Table 1). It had a low biodegradability, with a BOD_5/COD ratio of 0.19, where values lower than 0.25 indicate recalcitrant effluents (*15*). The similarity of these parameters between both collections was a coincidence; on both collection days the factory was producing the same products.

Table 1. Characterization of the meat and sausage processing factory wastewater that was used in the enzymatic prehydrolysis treatment

Parameter	1st collection	2nd collection	Method
pH	6.6±0.2	6.1±0.3	potentiometric
COD/(mg/L)	2706±267	3250±390	open reflux/titrimetric
BOD_5/(mg/L)	505±92	603±118	5-day incubation
BOD_5/COD	0.19	0.19	–
γ(OG)/(mg/L)	605±9	601±14	Soxhlet extraction

values are expressed as mean values±standard errors of the means
OG=oil and grease

Enzymatic prehydrolysis treatment

A packed-bed bioreactor was used for the enzymatic prehydrolysis (EPH) treatment (Fig. 1). It was aerated with an aquarium air pump, and the wastewater was fed at the bottom using a peristaltic pump. Magnetic stirring was used to keep the wastewater in the reservoir from settling, and a thermostat was used to maintain the temperature at 20 °C. Before use, it was corrected to pH=(7.1±0.1) with a 15 % (*m/V*) NaOH solution.

To fill the packed bed, the lipolytic fermented solid was defrosted at 7 °C for 24 h, and then placed in an incubator set at 25 °C for 6 h. A 5-gram sample was taken for lipase activity measurement, and 1.25 kg of moist solid was transferred into the bioreactor.
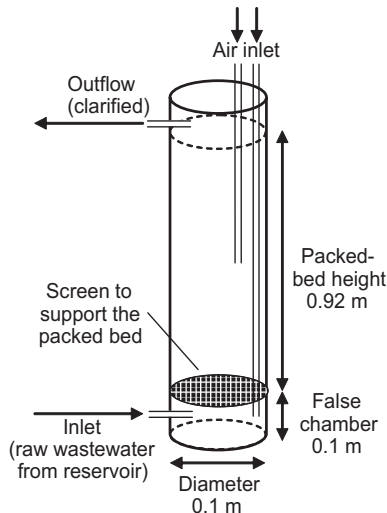
Fig. 1. Bioreactor for the enzymatic prehydrolysis and control test (not to scale)

The EPH experiments were carried out using five different values for the hydraulic residence time (HRT), which is related to the wastewater flow rate (Q/(L/h)) according to the following equation:

$$HRT = V/Q \qquad /1/$$

where V is the total volume of the packed bed (7.22 L).

The system started at an HRT of 120 h (Q=0.06 L/h), requiring 20 days for initial adaptation of *R. microsporus*. This HRT was maintained for a further 16 days. The HRT was then decreased, stepwise, to 96 h (Q=0.12 L/h), 72 h (Q=0.18 L/h), 48 h (Q=0.24 L/h) and, finally, 24 h (Q=0.30 L/h). Each of these HRT values was maintained for 16 to 17 days, which corresponds to a 2- to 3-day period for the stabilization of the outflow characteristics and 14 days of stable operation. The total time of operation of the EPH process was 101 days.

The dissolved oxygen level in the packed bed was measured daily using an oximeter (DM-4P, Digimed, São Paulo, Brazil). All measurements fell between 3.0 and 7.0 mg/L. Daily samples of fermented solid were taken from the top of the bed for measurement of lipolytic activity.

Daily measurements of pH, COD, BOD_5 and OG were made in the outflow at the top of the bioreactor. The removal fraction (of COD, BOD_5 or OG) was calculated for each HRT value, according to the Eq. 2:

$$\text{Removal fraction} = \frac{\gamma_0 - \gamma}{\gamma_0} \cdot 100 \qquad /2/$$

where γ_0 represents the COD, BOD_5 or OG concentration in the raw wastewater (mg/L) and γ represents the COD, BOD_5 or OG concentration in the outflow from the bioreactor (mg/L).

At the end of the EPH experiments, approx. 5 g of the fermented solid were removed from the packed bed and inoculated into two Petri dishes, one containing only PDA (39 g/L), the other containing PDA (39 g/L) and 100 mg/L of chloramphenicol. Both Petri dishes were incubated at 30 °C for 5 days.

A control experiment was undertaken to establish the influence of the lipase produced by *R. microsporus* in the EPH treatment. The bioreactor was filled with the same

3:1 mixture of sugarcane bagasse and triturated sunflower seed, but was not inoculated with *R. microsporus*. The control experiment was operated for a total of 37 days, using the highest and lowest HRT values of the EPH treatment: an HRT of 120 h, maintained for 21 days, followed by an HRT of 24 h, maintained for 16 days.

Determination of lipolytic activity

The lipolytic activity was measured directly in the fermented solid, using the titrimetric method with tricaprylin (C8) as substrate. After addition of 85 mg of the fermented solid to 20 mL of emulsion, a 0.05 mM NaOH solution was used to titrate the liberated acids, during 10 min, using a pH-Stat (718 Stat Titrino, Metrohm, Herisau, Switzerland) set at pH=7.0 and 37 °C. The emulsion contained Tris-HCl buffer 2.5 mM, $CaCl_2$ 2.0 mM, NaCl 150 mM, tricaprylin 63 mM and gum arabic 3 % (*m/V*). One unit (U) of activity represents the formation of 1 µmol of fatty acid per minute, under the assay conditions. The lipolytic activity was then expressed as units per gram of dried solid (U/g)±the standard error of the mean values obtained in replicate measurements.

Wastewater assays

All the assays done with the wastewater followed standard methods (*16*). COD was determined by the open reflux titrimetric method. BOD_5 was determined using a 5-day incubation at 20 °C; the seed culture was from a raw wastewater of a sewage treatment plant located in the SENAI school in Curitiba, Brazil. Soxhlet extraction with hexane was used to determine the OG content.

Results

Lipolytic activity of the fermented solid

The lipolytic activity of the solid produced by SSF was (70±7) U/g. After defrosting of the fermented solid, the activity decreased to (33±2) U/g. Activity losses after freezing and thawing are normal (*17–19*). The lipolytic activity in solid samples removed from the packed bed decreased rapidly during the first 10 days of the EPH experiment with the high-fat wastewater and then stabilized at a value of (6±3) U/g (Fig. 2). The most like-
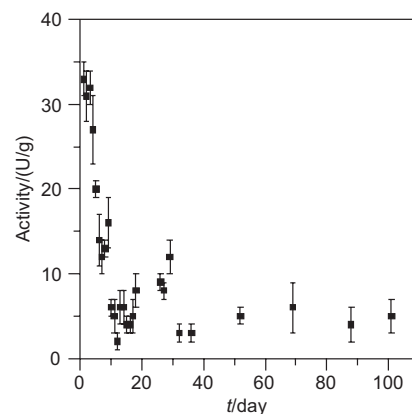

Fig. 2. Lipolytic activity per gram of dry solid in the top of the packed bed during enzymatic prehydrolysis experiment. The error bars represent the standard errors of the means

ly explanation for these results is that over the first 10 days the lipases produced during the fermentation were leached into the wastewater, but after this initial period, there was a balance between lipase production and leaching.

Enzymatic prehydrolysis of the high-fat wastewater

The removal fraction, between the inlet and the outlet of the packed bed, of COD, BOD_5 and OG is shown in Table 2 for both the EPH experiment and the control experiment. In interpreting these results, it is important to note that, although our main aim was to hydrolyze the fats in the effluent, the hydrolysis products can be metabolized further by the microorganisms present in the system, with the extent of this metabolism increasing as the HRT is increased.

A decrease in the HRT increases the loading rate of organic matter and consequently increases the COD and BOD_5 in the outflow. With an HRT of 24 h, the average BOD_5 in the outflow was higher for the EPH experiment (283 mg/L) than for the control experiment (187 mg/L). This is due to the greater hydrolysis of OG in the EPH experiment (average outflow OG of 22 mg/L) than in the control experiment (average outflow OG of 120 mg/L) and the fact that the hydrolysis products are more easily metabolized in the BOD_5 test than the fats from which they were derived.

Figs. 3a and 3b show the BOD_5/COD ratio of the outflow of both the EPH experiment and control experiment, at various HRT values. The outflow BOD_5/COD ratios for the EPH experiment were lower than 0.20 for the high HRT values (96 and 120 h), but between 0.40 and 0.59 for the lower HRT values (24 to 72 h). These results are related to the fate of the more easily biodegradable matter that is detected by the BOD_5 test: much more of this matter is removed at high HRT values than at low HRT values.

There was significant variability in the OG concentrations of the outflows of the EPH experiment (sample standard error of 22 mg/L) and control experiment

(sample standard error of 48 mg/L) for the various HRT values that were used (Figs. 3c and 3d). However, the outflow from the EPH experiment had lower OG levels (average of 28 mg/L, standard error of the mean of 3 mg/L) than did that of the control experiment (average of 113 mg/L, standard error of the mean of 10 mg/L), presumably due to the presence, in the EPH experiment, of the lipase produced by *R. microsporus*.

A comparison between the BOD_5/COD ratio of the outflow (Figs. 3a and 3b) and the OG of the outflow (Figs. 3c and 3d) shows that the differences in the BOD_5/COD ratios that were obtained when the EPH experiment and control experiment were operated at the same HRT are explained by the differences in the amount of OG that was hydrolyzed. It should be noted that for both HRT values at which the control experiment was run (*i.e.* 24 and 120 h), the outflow COD values of the control experiment and the EPH experiment are similar to each other, so the differences between the two BOD_5/COD ratios in the outflows are related not to the overall quantity of organic material but rather to the availability of that organic material to the microorganisms. At the HRT of 24 h, the EPH experiment (Fig. 3a) had a higher BOD_5/COD ratio than did the control experiment (Fig. 3b). This occurred because the presence of the lipolytic activity in the EPH experiment led to hydrolysis of the fats in the wastewater (Fig. 3c), whereas in the control experiment the fats remained largely unhydrolyzed (Fig. 3d). The fatty acids are much more easily biodegradable in the subsequent BOD_5 test than are the original fats. In a similar manner, the high BOD_5/COD ratio of the outflow from the EPH experiment for the HRTs of 48 and 72 h can be attributed to the hydrolysis of the fats by the lipolytic solids.

On the other hand, at the HRT of 120 h, the outflow from the EPH experiment (Fig. 3a) had a lower BOD_5/COD ratio than did that of the control experiment (Fig. 3b). This occurred because this residence time was sufficiently high to allow not only hydrolysis of the fats within the EPH experiment but also consumption of the released fatty acids.

Table 2. Removal fraction of COD, BOD_5 and oil and grease (OG) during the enzymatic prehydrolysis (EPH) experiment and in the control experiment for different hydraulic residence times (HRT)

Experiment	HRT	COD		BOD_5		BOD_5/COD	OG	
	$\frac{\text{HRT}}{\text{h}}$	Outflow value	Removal fraction	Outflow value	Removal fraction		Outflow value	Removal fraction
		mg/L	%	mg/L	%		mg/L	%
control	120	209±51	93.0	68±39	87.8	0.33	106±66	79.0
EPH	120	225±20	91.5	25±5	95.6	0.11	21±25	95.9
	96	288±32	90.3	44±10	92.1	0.15	49±20	90.3
	72	378±28	87.3	183±26	67.0	0.48	19±20	96.2
	48	461±38	84.5	237±27	57.3	0.51	28±19	94.5
	24	515±17	82.7	283±17	49.0	0.55	22±11	95.7
control	24	520±52	82.6	187±26	66.3	0.36	120±14	76.2

Results are expressed as the average value±standard error of the mean. For the calculation of averages, the adaptation times of 2 to 3 days after the switch to each new HRT value were not considered
The removal fractions were calculated taking the average value of COD, BOD_5 and OG for each HRT value
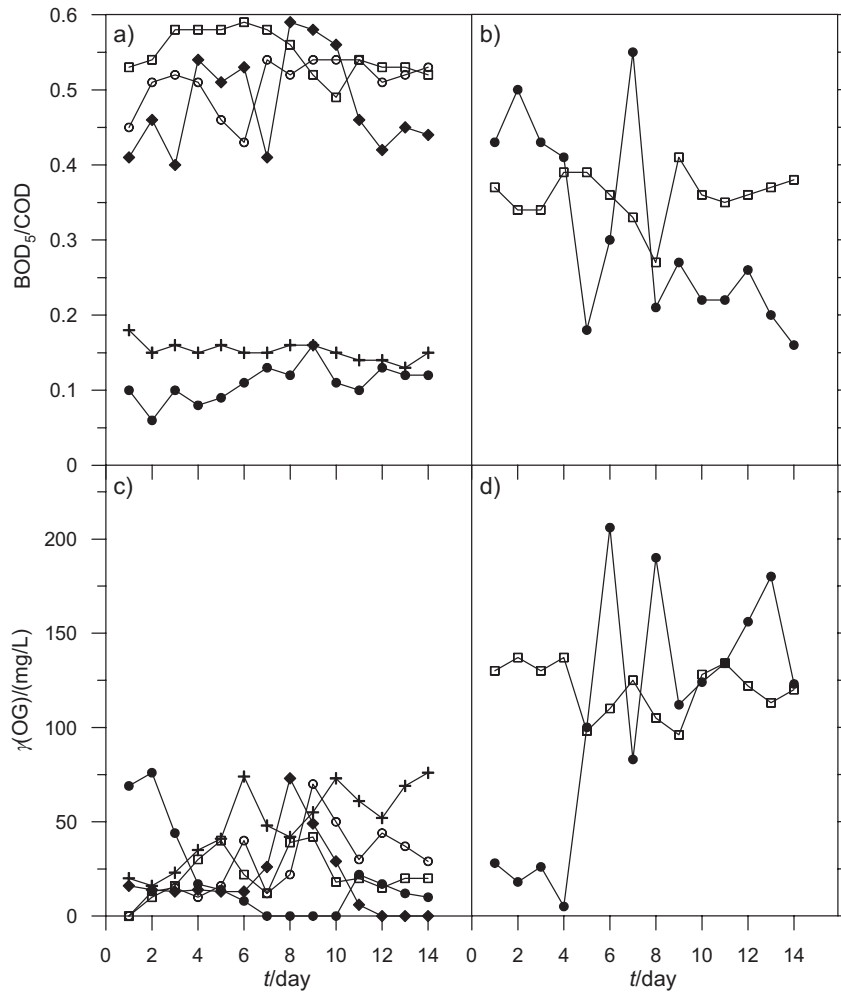
Fig. 3. Effect of HRT on the BOD_5/COD ratio and OG levels in the outflow of the packed-bed bioreactor. The top graphs show BOD_5/COD levels in: a) the enzymatic prehydrolysis (EPH) experiment and b) the control experiment. The average BOD_5/COD ratio in the raw wastewater fed to the packed-bed bioreactor was 0.19. The bottom graphs show OG levels in: c) the enzymatic prehydrolysis (EPH) experiment and d) the control experiment. The average OG in the raw wastewater fed to the packed-bed bioreactor was (603±3) mg/L. Key: HRT values of (●) 120, (+) 96, (◆) 72, (○) 48 and (□) 24 h. For each HRT, the results are plotted over the 14-day period of stable operation (for details, see *Enzymatic prehydrolysis treatment* in the Materials and Methods section)

Samples of the fermented solid were removed from the bioreactor on the 96th day of the EPH experiment, plated on Petri dishes containing PDA, with and without chloramphenicol (which inhibits bacterial growth), and incubated for 5 days at 30 °C. There were no visual differences in the growth between the plates with chloramphenicol and without it (Fig. 4a): in both cases a brownish-gray mycelium filled the whole height of the Petri dish, with this mycelium containing short sporangia and hemispherical columella (Fig. 4b) that are character-
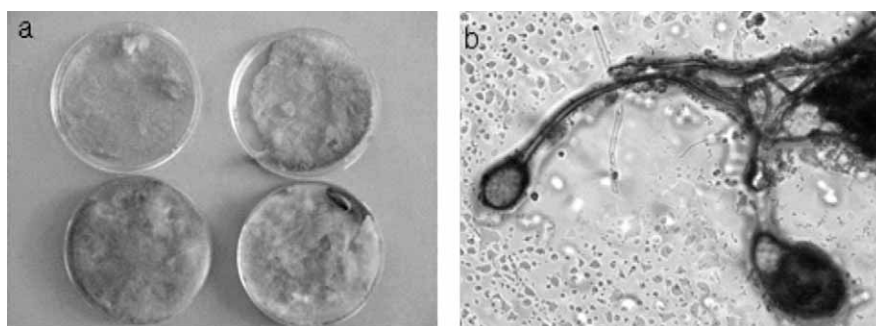

Fig. 4. Growth of *Rhizopus microsporus* after inoculation of the fermented solid from the packed-bed bioreactor onto PDA: a) open Petri dishes (top and bottom) after a 5-day incubation at 30 °C. The plate with PDA and chloramphenicol is on the left, while the plate with PDA and no chloramphenicol is on the right. Both plates contain brownish-gray mycelium typical of *R. microsporus*; b) micrograph, using optical microscopy at 400×, showing short sporangia and hemispherical columella, which are typical of *R. microsporus*

istic of *R. microsporus* (20,21). This shows that *R. microsporus* was able to compete with the endogenous bacteria of the wastewater. Further, it was able to inhibit bacterial growth, since there were no bacterial colonies on the plate incubated without chloramphenicol. Given that *R. microsporus* remained viable throughout the EPH experiment, it is most likely that the higher hydrolysis of OG in this experiment compared to the control experiment is due to its production of lipolytic activity.

The same inoculation in Petri dishes was done for the control experiment, also using one plate with PDA and another one with PDA and chloramphenicol (Fig. 5). After 5 days of incubation at 30 °C there was no growth of *R. microsporus* on either plate: only bacterial colonies

Fig. 5. Growth of endogenous microflora of the control treatment after a 5-day incubation at 30 °C. The plate with PDA and chloramphenicol is on the left, while the plate with PDA and no chloramphenicol is on the right. Neither plate shows fungal growth typical of *R. microsporus*, although bacterial colonies and fungal mycelia originating from the endogenous microflora are visible

and the formation of fungal mycelia originating from the endogenous microflora were observed. The poorer removal of OG obtained in the control experiment suggests that this microflora did not produce a high lipolytic activity.

Discussion

The current work extends a previous study into the use of fermented solids, produced using *Rhizopus microsporus*, to treat high-fat wastewaters (13). The advantage of our present study is that it uses real effluents: much of the previous work was done with an artificial system involving sterilized effluents, in order to study the performance of the fermented solid in the absence of interference from the endogenous microflora of the effluent. The current work makes two important contributions. Firstly, it shows that *R. microsporus* can maintain its lipolytic activity for long periods, even in the presence of the endogenous microflora. Secondly, it represents the first time that a packed-bed bioreactor has been used for the enzymatic pretreatment step.

Potential of Rhizopus microsporus for enzymatic pretreatment of high-fat wastewaters

Previous studies of the addition of fermented solids to pretreat unsterilized effluents have involved various fungal and bacterial strains and have used short treat-

ment times of up to 120 h (13). We have shown, for the first time, that the microorganism (in our case, a fungus) can survive and maintain lipolytic activity within the fermented solids for three months. In the current work the experiment was terminated after 101 days; future studies should investigate just how long the lipolytic activity can be maintained in the solid. The longer it can be maintained, the lower will be the costs associated with the production of the fermented solid. The aeration of the bioreactor may have helped *R. microsporus* to maintain itself, although future studies should also investigate whether there is a real need for aeration. If it is not necessary, then the operational costs of the packed-bed will be reduced significantly.

The use of a packed-bed bioreactor

The current work represents the first time that the enzymatic prehydrolysis of a high-fat wastewater has been undertaken using a packed-bed bioreactor filled with fermented solid. The advantage of this type of bioreactor is that it allows a high concentration of lipolytic activity. In contrast, previous studies (10,11) have involved the addition of relatively small amounts of fermented solid: in one study the fermented solid loading was 5 % (m/V), but in most studies it was 1 % (m/V) or less (Table 3).

Potentially, the packed-bed bioreactor could be used in one of two modes, depending on the particular circumstances. Firstly, it could be used with a short residence time for pretreatment, with the pretreated effluent then being sent to a traditional anaerobic digestion or activated sludge process (8,10,12,14). The aim would not be to remove COD, but rather to remove OG and to increase the BOD_5 and, consequently, increase the biodegradability. The residence time in the packed-bed bioreactor would need to be optimized in order to achieve this. On the basis of our results, the HRT could be as low as 24 h: at this residence time we obtained a reduction in the OG level from approximately 600 mg/L at the inlet to less than 25 mg/L at the outlet of the bed. Such OG levels are quite acceptable for wastewaters entering conventional treatment processes (4,5,8,10–12,14). Importantly, at this HRT the biodegradability of the pretreated effluent was excellent, with a BOD_5/COD ratio of above 0.5.

Secondly, the packed-bed bioreactor could be used with a long residence time (of 96 to 120 h) as a single-step treatment process, as removal of over 90 % of the BOD_5, COD and OG can be achieved. At these residence times the effluent very nearly met the required parameters for the emission of effluents in the state of Paraná, Brazil (CEMA 070/2009) (22). The only parameter that was not met in these cases was the COD limit for emission of 200 mg/L. However, a low-cost and simple treatment step, with low sludge formation, such as a maturation lagoon, wetlands or coagulation, would bring the COD to below this level. Also, an HRT higher than 120 h within the packed bed should also be investigated, since it might be sufficient to bring the COD level under the acceptable limit.

Future studies should be aimed at investigating important questions regarding the packed-bed bioreactor. Firstly, it needs to be determined whether it is possible to produce a solid material that is based wholly on resi-

Table 3. Characteristics and efficiency of enzymatic prehydrolysis systems treating high-fat wastewaters

Source and description of system	Fermented solid to wastewater ratio*/%	Lipase producer	Maximum COD removal	Maximum BOD_5 removal	Maximum OG removal
Present work: 0.06 to 0.3 L/h of continuous flow of wastewater into a packed-bed loaded with 1.25 kg of moist fermented solids.	–	*Rhizopus microsporus*	92 % without post treatment, at HRT of 120 h	96 % without post treatment, at HRT of 120 h	96 % without post treatment, at HRT of 120 h
Durli (9): 24- to 48-hour batches. Flasks with 0.3 L of wastewater. Small fermented solid portions added directly under stirring.	0.1	*Burkholderia cepacia*	100 % after extended aerobic treatment	100 % after extended aerobic treatment	82 % without post treatment
Valladão et al. (8): 22-hour batches. Flasks with 0.4 L of wastewater. Small fermented solid portions added directly under stirring.	0.1 to 1	*Penicillium restrictum*	83 % after anaerobic treatment	not evaluated	not evaluated
Damasceno et al. (14): 0.14 L/h of continuous flow. Laboratory-scale activated sludge system. Small fermented solid portions added directly under stirring.	0.1	*Penicillium restrictum*	90 % after aerobic treatment	not evaluated	not evaluated
Rigo et al. (10): 24-hour batches. Flasks with 0.09 L of wastewater. Small fermented solid portions and Lipolase 100T (Novozymes) added directly under stirring.	0.1 and 5	*Penicillium restrictum*	85 % after anaerobic treatment	not evaluated	not evaluated
Rosa et al. (12): 24-hour batches. Reservoir for 18 L of wastewater. Small fermented solid portions added directly under stirring.	0.1	*Penicillium* sp.	90 % after anaerobic treatment	not evaluated	not evaluated
Alberton et al. (13): 12- to 120-hour batches. Flasks with 0.07 L of wastewater. Small fermented solid portions added directly under stirring.	0.3	*Rhizopus microsporus*	not evaluated	not evaluated	91 % without post treatment

*by mass per volume; OG=oil and grease

dues, such that it is cheaper to produce than our mixture of solids (which contains ground sunflower seeds), but which allows the long term maintenance of a lipolytic activity that is equivalent to that obtained in the current work, or even higher. Secondly, the samples for determination of lipolytic activity of the fermented solids in the packed bed were removed from the top of the bed (near the outlet of the bed); the distribution of lipolytic activities along the bed should be studied. Thirdly, it needs to be determined whether, after extended use, large clumps of fungal biomass, or a network of fungal biomass and fermented solids will be intermittently liberated from the packed bed and appear in the outflow, analogous to the 'sloughing' phenomenon that occurs with trickling filters and rotating disk reactors. Even if this does happen, it should not pose a critical problem, since any such solid material should be removed in subsequent sedimentation steps.

Comparison of the packed-bed bioreactor with other enzymatic pretreatment systems

Table 3 summarizes the main results of the current work and compares them with recent studies of enzymatic pretreatment systems involving lipolytic fermented solids. An important aspect of our work is that we demonstrated that the treatment with the fermented solids is, in itself, able to remove a significant amount of COD, BOD_5 and OG. Many authors have not tested for these parameters after the treatment with fermented solids, but rather only after the wastewater had passed through a subsequent classical aerobic or anaerobic treatment process. At an HRT of 120 h, the removal of COD, BOD_5 and OG in our packed-bed bioreactor was comparable to that obtained by other authors with two-step processes that involve both the pretreatment and classical treatment (Table 3).

Alberton et al. (13) used the same fermented solid as that used in the present study (i.e. produced by *R. microsporus* with the same mixture of solid substrates). They added 0.3 % (m/V) of fermented solid to sterilized and unsterilized effluents containing 1500 mg/L of OG. With unsterilized effluent, a 72-hour treatment was necessary to reduce the BOD_5 by 90 % (from an initial value of 4500 mg/L). However, even with this long treatment time, the final OG level was still around 250 mg/L. Although we used a lower initial OG level of 600 mg/L, with a residence time of 72 h, the outflow OG level was maintained below about 40 mg/L. In future studies it will be necessary to test our packed-bed bioreactor with wastewaters with higher OG levels in order to be able to make a more direct comparison.

Conclusion

We have shown, for the first time, that a packed-bed bioreactor filled with fermented solid (produced by solid-state fermentation) has potential to be used for the enzymatic treatment of high-fat wastewaters: our laboratory-scale packed-bed was able to reduce BOD_5, COD and OG levels of a wastewater from a meat and sausage processing factory by over 90 % and residual lipolytic activity was maintained in the fermented solid within

the bioreactor for at least 100 days. Further studies are necessary to explore the potential of the packed-bed system further, characterizing and optimizing its performance, in order to provide the basis for scale-up to a pilot-scale unit.

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