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Pre-treatment of high oil and grease pet food industrial wastewaters using immobilized lipase hydrolyzation

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Abstract

Wastewaters generating from pet food industries contain high concentration of oil and grease (O&G), which is difficult to treat through conventional biological treatment systems. In this study, the hydrolysis of O&G originating from pet food industrial wastewater was evaluated. *Candida rugosa* lipase was immobilized in calcium alginate beads and applied in the hydrolysis experiment. Results showed that approximately 50% of the O&G was hydrolyzed due to the enzyme activity. A significant increment in COD and VFA production was also observed. The immobilized lipase activity was confirmed with *p*-nitrophenyl palmitate (*p*NPP) before and after O&G hydrolysis. During the 3-day experiment, approximately 65% of the beads were recovered and after the hydrolysis, approximately 70% of the enzyme activity remained in the beads. This study shows the potential of immobilized lipase as a pre-treatment step in biological treatment of pet food manufacturing wastewater.

Keywords: Hydrolysis; Immobilized lipase; Alginate beads; Oil and grease; Pet food wastewater

1. Introduction

Wastewaters from pet food manufacturing industries have high concentration of oil and grease (O&G) and very high chemical oxygen demand (COD) levels, which are difficult to treat through biological treatment system mainly due to slow biodegradability kinetics [1]. In aerobic processes, O&G also lessen the oxygen transfer efficiency. Particularly, in the activated sludge process, O&G increases the filamentous actinomycete *Nocardia amarae*, which cause the formation of scum and foams [2]. In anaerobic processes, the characteristics of oily wastewater lead to many challenges. Sludges with diverse characteristics with poor activity can develop and have foam on the surface of the water. This may also affect the biomass losses with effluent, decreasing biomass quantity within the reactor and hence the efficiency of the treatment system. In addition, O&G can be adsorbed on the surface of the anaerobic sludge, which may limit the transport of soluble substrates to the biomass. This leads to reduction in rate of substrate conversion. At lower temperatures, fats may solidify and create operational problems such as clogging and producing unpleasant odors [3]. Therefore, it is necessary to identify and apply alternative or modified treatment processes for treating oily wastewater.

Major constituents of animal fat are triglycerides consisting of straight-chains of fatty acids (e.g. palmitic and oleic acid) attached, as esters, to glycerol [4]. The component fatty acids of edible fats vary considerably depending on the source of wastewater. They may be saturated or unsaturated, can vary in chain length and number of carbon atoms; may be free, dispersed or emulsified. Moreover, antioxidants and other chemicals used for floor cleaning may modify the constituents of the wastewater considerably.

Lipases are enzymes or biocatalysts, which have the ability to catalyze cleavage of carboxyl ester bonds in tri-, di- and monoacylglycerols [5]. Lipases cleave ester bonds of triacylglycerols with the consumption of water molecules (hydrolysis). There are several research studies available on the treatment of oily wastes using lipase [3,6–9]. However, most of these studies were focused on pre-treatment of wastewater with synthetically added fats. Moreover, soluble lipase was used for hydrolysis. Lipase from *Candida rugosa* (formally *Candida cylindracea*) is a microbial enzyme produced by fermentation of yeasts [10]. It was extensively used for oil hydrolysis [10,11–14] because it is one of the most attractive, commercially available lipases

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and has the ability to liberate all types of acyl chains. However, application of the lipase to real industrial wastewater has not been reported yet.

Lipases are generally soluble and unstable, hence can be used only once in solutions. In addition, commercially available lipases are costly. These problems can be easily overcome by immobilization of enzymes. Immobilized enzymes have the advantages of multiple usage, controlled reactions, and mechanical stability. However, loss of enzyme activity during the immobilization procedure [14] and during the reaction is the main hurdle for widespread commercialization. Immobilization of enzymes can be accomplished in many ways. The most important methods are immobilization by binding (physical adsorption, covalent attachment or cross-linking) and immobilization by physical retention (matrix entrapment or membrane enclosure). Alginate, nontoxic polysaccharide, is widely used as support material because it is cheap, and has good gelling properties [15].

In this study, hydrolysis of O&G from pet food industrial wastewaters using lipase was evaluated. *C. rugosa* lipase immobilized in calcium alginate beads were used in the hydrolysis studies. At first, immobilized alginate beads were produced at different conditions. Optimum ratio of lipase to sodium alginate was identified for producing active alginate beads. Then several trials were carried out to quantify the optimum bead dosage to the real wastewater, by varying the amount of beads. Finally, batch hydrolysis experiments were conducted for both autoclaved and non-autoclaved wastewater at various conditions. The immobilized lipase activity was confirmed with *p*-nitrophenyl palmitate (*p*NPP) before and after fat hydrolysis. The investigations are discussed below.

2. Materials and methods

2.1. Chemicals

The lipase used in this experiment was from *C. rugosa* (EC 3.1.1.3) sold by Sigma–Aldrich Pvt. Ltd. (St. Louis, MO) with activity of 890 U/mg. Sodium alginate was also purchased from Sigma. All the other chemicals were purchased from VWR, Canada. All the chemicals used in this experiment were reagent grade and used without further purification.

2.2. Immobilization protocol

C. rugosa lipase immobilized in calcium alginate beads were produced as discussed elsewhere [15,16] with some modifications. Briefly, 2 g of lipase and 2 g of sodium alginate were dissolved in 100 mL of deionized water and mixed with a magnetic stirrer thoroughly for 4–5 h to get uniform solution. The mixture was then extruded drop wise from a syringe by gravity into $CaCl_2$ (180 mM) solution. The fresh beads were slowly mixed for 30 more minutes to harden the surface. Then the beads were filtered using vacuum filtration and washed with phosphate buffer solution (pH 7.2) and deionized water. Finally, the beads (ca. 5 g) were stored at 4 °C for further use. The amount of free lipase in the beads, estimated by measuring the initial weight of lipase (2 g) in immobilization solution used for each batch of immobilization and the final dry weight of beads (5 g), was about 0.4 g lipase/g of beads. The protein content in the used hardening solution and washed deionized water was negligible. Therefore, almost complete immobilization could be attained using this technique.

2.3. Lipase activity

The lipase activity of immobilized lipase was measured as previously described [17]. In this assay, the release of *p*-nitrophenol (*p*NP) from *p*NPP was measured spectrophotometrically at 410 nm. Required reagents were prepared using the following formula. The solution 1 was prepared by dissolving 30 mg of *p*NPP in 10 mL 2-propanol and the solution 2 was prepared by mixing 0.4 g of Triton® X-100 and 0.1 g of Gum Arabic in 90 mL of phosphate buffer at pH 7.2. Solution 1 was added drop-wise to solution 2 with continuous stirring to get a milky suspension and preheated to 35 °C before use. The specific activity of lipase was determined by mixing 0.3 mg of free lipase (1–2 beads) with 2.7 mL assay medium.

The lipase activity was calculated using the following formula:

$$U = \frac{V}{m\varepsilon d(\Delta A)} \quad (1)$$

where; U is the unit lipase activity (U/mg), assessed by measuring the increase in absorbance of *p*NP; V the volume of substrate solution (ml)—2.7 ml; m the mass of lipase (mg)—0.3 mg; ε the extinction coefficient of *p*NP at 410 nm (1/mmol cm) – (15 mmol cm)$^{-1}$; d the light path of cuvette (cm); ΔA the change of absorbance per minute (nm/min).

For pet food wastewater, animal fat emulsion method was used to determine the immobilized lipase activity by measuring the release of free fatty acids by titration [18]. One unit of enzyme activity (U) was defined as the amount of enzyme, which liberated 1 μmol of free fatty acid/min under the assay conditions. Relative activity was defined as the ratio of activity at any condition to the maximum activity at optimum assay conditions.

2.4. Protein assay

Protein content was measured according to Bradford method [19]. Briefly, sample volume of 0.2 mL was added to 0.8 mL diluted reagent (coomassie brilliant blue G-250) in disposable cuvette and thoroughly mixed before measuring the absorbance at 595 nm using BSA as standard protein.

2.5. Bead characterization

The density, average mass of bead and average diameter were measured accurately for the immobilized beads. The particle size distribution was measured by using particle size analyzer (Mastersizer, Spectra Research Corporation, Ont., Canada). Repeat measurements showed that the bulk density was 925 kg/m^3, solid density was 1427 kg/m^3, average mass of a bead was 0.95 mg and the volume-weighted average bead diameter was 1 ± 0.5 mm.

Table 1
Raw wastewater characteristics

Parameter	Range	Average	Unit	Method
Total chemical oxygen demand (TCOD)	74000–100960	87480	mg/L	Hach
Soluble chemical oxygen demand (SCOD)	37500–64980	51240	mg/L	Hach
Oil and grease (O&G)	52000–114000	83000	mg/L	Gravimetric
Total suspended solids (TSS)	68500–96000	82250	mg/L	Standard methods
Volatile suspended solids (VSS)	41600–60900	51250	mg/L	Standard methods
Ammonia	680–1485	1353	mg/L	Hach
Phosphate	240–355	286	mg/L	HPLC
Nitrate	26–54	40	mg/L	HPLC
Sulfate	285–359	322	mg/L	HPLC
Alkalinity	2600–3800	3200	mg/L	Standard methods
Volatile fatty acid (VFA)	4000–5800	4900	mg/L	GC
pH	6.4–7.0	6.7		pH meter

2.6. Raw wastewater

The raw wastewater was collected from a local pet food manufacturer. The plant generates around 60 m^3/d of wastewater, which has very high COD, suspended solids and O&G (Table 1). Wastewater was produced mainly from production line and equipment and floor cleaning operations. The processing operations include freezing of meat by-products for storage prior to processing into feed, coarse grinding of meat by-products, washing of chicken viscera to remove chicken manure, cooking chicken viscera wash water to remove fat and tallow, centrifuging chicken viscera wash water to remove meat particles and high melting fats, cooking meat by-products, emulsifying (fine grinding), mixing feed ingredients and freezing finished feed [1].

Since the raw wastewater has very high COD, suspended solids and O&G, diluted wastewater (1:6) was used during all the experiments.

2.7. Batch experimental set-up

The hydrolysis experiments were carried out as follows. In six-1 L flasks, 100 mL raw wastewater was taken and diluted to 600 mL. Three flasks (flask # 1–3) were filled with autoclaved (121 °C, 20 min) wastewater to differentiate the effect of existing microorganisms during the hydrolysis (Table 2). Then beads were added to four flasks (except #1 and #4) at two different concentrations (#2 and #5 with 0.6 g and #3 and #6 with 1.2 g). All the flasks were adjusted to a pH of 7.2 using sodium bicarbonate and kept in an orbital shaker at 150 rpm. The temperature was maintained at 35 °C for 3 days. Each day, 10 mL of samples were taken for analysis. After 3rd day, the flasks were taken out from the shaker and left to settle. Only batches with lipase were duplicated to confirm reproducibility.

2.8. Analytical methods

Soluble chemical oxygen demand (SCOD), volatile fatty acid (VFA), and oil and grease (O&G) were measured daily for 3 days. The pH was adjusted to 7.2 (optimum for lipase activity) daily. Analysis of O&G and SCOD were carried out as per standard methods for the examination of water and wastewater [20]. The O&G was analyzed by taking known volume of sample in a pre-weighed dark bottle and was acidified to a pH of 2 using 1:1 H_2SO_4. Then 30 mL of hexane was added and transferred to a centrifuge tube for centrifugation for 5 min at approximately 2400 rpm. The centrifuged material was transferred to the separatory funnel and the solvent layer was drained through filter paper to the distilling flask. The filter paper was pre-rinsed with hexane and Na_2SO_4 was added to remove water. All the remaining aqueous layers were be extracted with 30 ml solvent twice as described above. Finally, the solvent in the distilling flask was distilled at 85 °C and the remaining O&G was dried with N_2 gas. The final weight was taken for O&G calculation.

Samples were filtered by a 0.45-micron glass fiber filter paper for the determination of SCOD and VFA. VFA was measured using a gas chromatograph (Varian 8500, Varian Inc., Toronto, Canada) equipped with FID detector (split/splitless injection

Table 2
Optimum ratio of lipase and sodium alginate for immobilization

Alginate (g)	Lipase (g)	Final weight (g)	Lipase in beads (g lipase/g beads)	Activity (U/mg)	Standard deviation
1	2	4.2	0.48	641	20
2	2	5.1	0.39	763	10
3	2	6.0	0.33	705	19
4	2	6.4	0.31	683	14
5	2	7.8	0.26	649	32
2	1	4.1	0.24	695	8
2	2	4.9	0.41	776	17
2	3	6.2	0.48	692	64
2	4	6.5	0.62	673	14

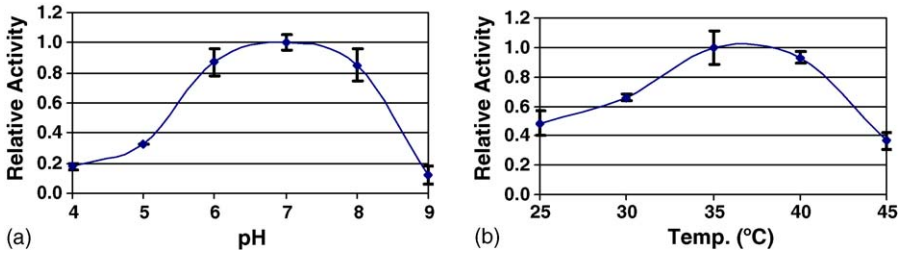

Fig. 1. Effect of pH and temperature on immobilization of lipase.

system with auto-sampler). The analytical column (fused silica type) was 30 m length, 0.32 mm internal diameter and 5 μm thickness. Lipase activities were measured before and after the hydrolysis experiment using the lipase assay described in the Section 2.3.

3. Results and discussion

Various experiments were conducted to identify the optimum ratio of lipase and sodium alginate for producing alginate beads. In each immobilization protocol, different ratios were selected and for each set, the enzyme activity was estimated using *p*NPP as substrate. The optimum ratio of lipase and sodium alginate on mass basis was 1:1 (Table 2) and the concentration of lipase was 20 g lipase/L of solution which is comparable with the results obtained previously [16]. In addition, the amount of lipase in the beads was about 0.4 g lipase/g beads. The effect of pH and temperature on the activity of immobilized lipase usually represented in the form of optimum curves where activity is given as relative activity. Fig. 1 shows the relative activity at different pH and temperature and the optimum pH and temperature were found to be similar to free lipase (pH 7.2 and temperature = 35 °C). To quantify the optimum bead dosage to the oily wastewater, several trials were carried out by varying the amount of beads (5–50 mg) and the lipase activity was carried out using animal fat emulsion method. As seen from Fig. 2, the maximum rate of hydrolysis was obtained when the dosage was between 0.5 and 1.5 g of beads/L of wastewater.

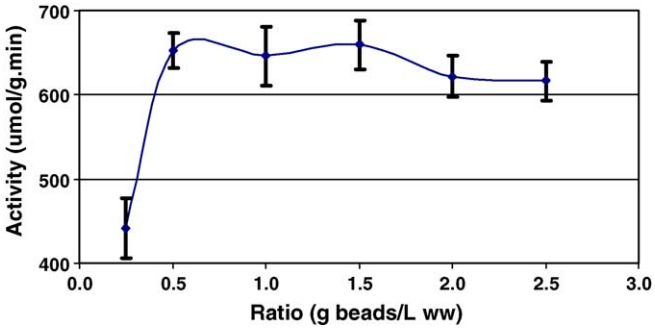

Fig. 2. Effect of beads to substrate ratio on lipase activity.

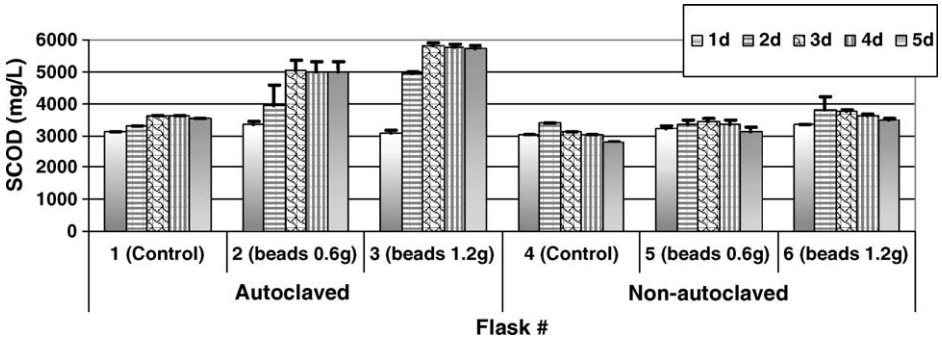

Fig. 3. Daily changes in SCOD during the hydrolysis.

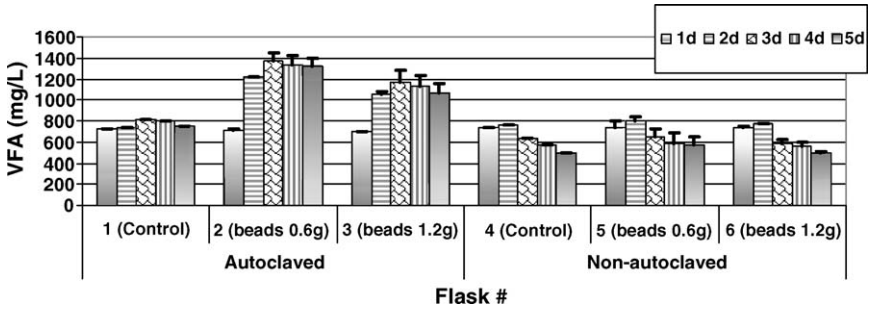

Fig. 4. Daily changes in VFA during the hydrolysis.

In order to assess kinetics of hydrolysis, a hydrolysis experiment was conducted for 5 days. The daily measurement of COD and VFA showed that after 3rd day, the hydrolytic changes in COD and VFA were not significant (Figs. 3 and 4). Hence, 3 day-period was selected for all the experiments. In the hydrolytic experiment, all the flasks were shaken vigorously and left to settle after 3rd day. Most of the flasks had three distinct layers namely float, clear liquid, and solids. Float, the top and brownish layer, was made out of O&G. Clear liquid, the middle and light yellowish color layer, was water with dissolved materials. Solids, the bottom and heavy layer, were made of silt and beads surrounded by grease. The controls (#1 and #4) were showing higher volume of floats (Fig. 5a), nearly 10% by volume, which are O&G in the raw wastewater. All the other flasks (#2–3 and #5–6), which were enzyme treated wastewater were showing only less than 2% of floats. In addition, these flasks had solids settled at the bottom of the flasks. The autoclaved samples were showing (Fig. 5b) lesser solids (0.8–1.8%) than non-autoclaved samples (1.7–2.5%). This is probably due to thermal effect where the heat might have dissolved the floats before hydrolysis. From these visual observations, it can be predicted that the lipase is converting the fats into soluble products.

The measurement of O&G is one of the important parameters in hydrolysis reactions. It gives an idea about how much O&G have been converted during the hydrolysis. In this experiment, the final O&G concentrations of each sample were compared with the control. Samples for O&G analysis were taken after vigorous mixing. The results (Table 3) showed that approximately 40–60% reduction in O&G occurred during the hydrolysis. The autoclaved samples (#2–3) showed higher overall O&G reduction than non-autoclaved samples (#5–6). This might be partial hydrolyzation due to autoclave temperature before the enzymatic hydrolysis. However, the O&G reductions for all the lipase-loaded samples were similar.

Percentage change in SCOD, VFA and O&G after 3 days in all the flasks is depicted in the Fig. 6. It should be noted that, in general, SCOD and VFA's are expected to increase while O&G decreases due to the action of lipase. The controls (#1 and #4) were showing lower SCOD and VFA change compared to other flasks. The enzyme treated, autoclaved samples (#2–3) were showing increment in SCOD, VFA but reduction in O&G where enzyme treated, non-autoclaved samples (#5–6) were showing increment in SCOD but reduction in VFA and O&G. Particularly, flasks #5 and 6 which was autoclaved samples with 1.2 g of bead dosage gave the highest SCOD increment (80–90%).

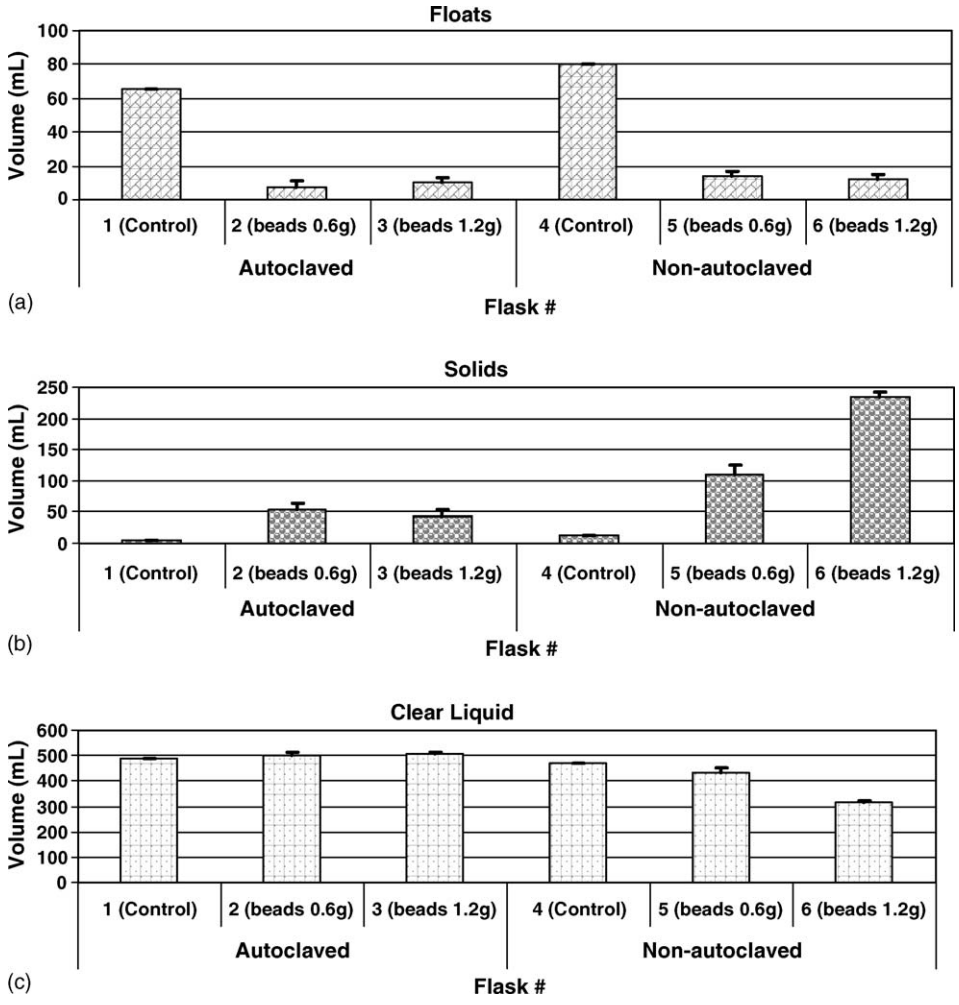

Fig. 5. Influence of different amount of beads and the effect of sterilization on the hydrolysis (a) solids (b) clear liquid (c) floats.

Table 3
Initial experimental conditions and O&G reduction during the hydrolysis

Flask #[a]	Raw ww (ml)	Beads (g)	Final V (ml)	Autoclaved	O&G (g/L)			O&G red (%)[b]
					Initial	After autoclaved	Final	
1	100	0.00	600	Y	9.83	9.04	9.04	8.0
2	100	0.63 ± 0.01	600	Y	9.83	9.04	4.56 ± 0.51	53.6 ± 5.18
3	100	1.23 ± 0.01	600	Y	9.83	4.4	4.46 ± 0.45	54.6 ± 4.60
4	100	0.00	600	N	9.83	9.83	9.63	2.0
5	100	0.63 ± 0.01	600	N	9.83	9.83	5.40 ± 0.18	45.1 ± 1.87
6	100	1.20 ± 0.03	600	N	9.83	9.83	5.32 ± 0.62	45.8 ± 6.33

[a] 1–3: autoclaved, 4–6: non-autoclaved, 1&4: control, 2&5: 0.6 g beads, 3&6: 1.2 g beads.
[b] O&G reduction = $100 \times (O\&G_1\text{-}O\&G_i)/O\&G_1$, where 1 = flask # 1 and i = 2–6.

This is because the insoluble fat particles were converted into soluble products by both enzyme hydrolysis and thermal effect, which increased the SCOD. On the other hand, flasks #5 and 6 gave only 10% SCOD increment which can be attributed to microbial activity which degraded products of hydrolysis during the 3 day experiment. Similar explanations could be given to other flasks (#2 and #5). In all the autoclaved samples, SCOD increased gradually (Fig. 3) from the 1st day to 3rd day where the non-autoclaved samples did not show the same phenomenon.

Lipase hydrolyzes triacylglycerols into VFA and glycerol. Though the reaction converts O&G in to long chain fatty acids (LCFA), they also produce some short chain volatile fatty acids (VFA). Therefore, measurement of VFA is an important parameter in this experiment. In general, all the autoclaved samples showed VFA increment whereas others did not (Fig. 6b). Moreover, in autoclaved samples, VFA increased gradually (Fig. 4) due to hydrolysis, which released free VFA. Although the non-autoclaved samples showed VFA increment on the 2nd day, due

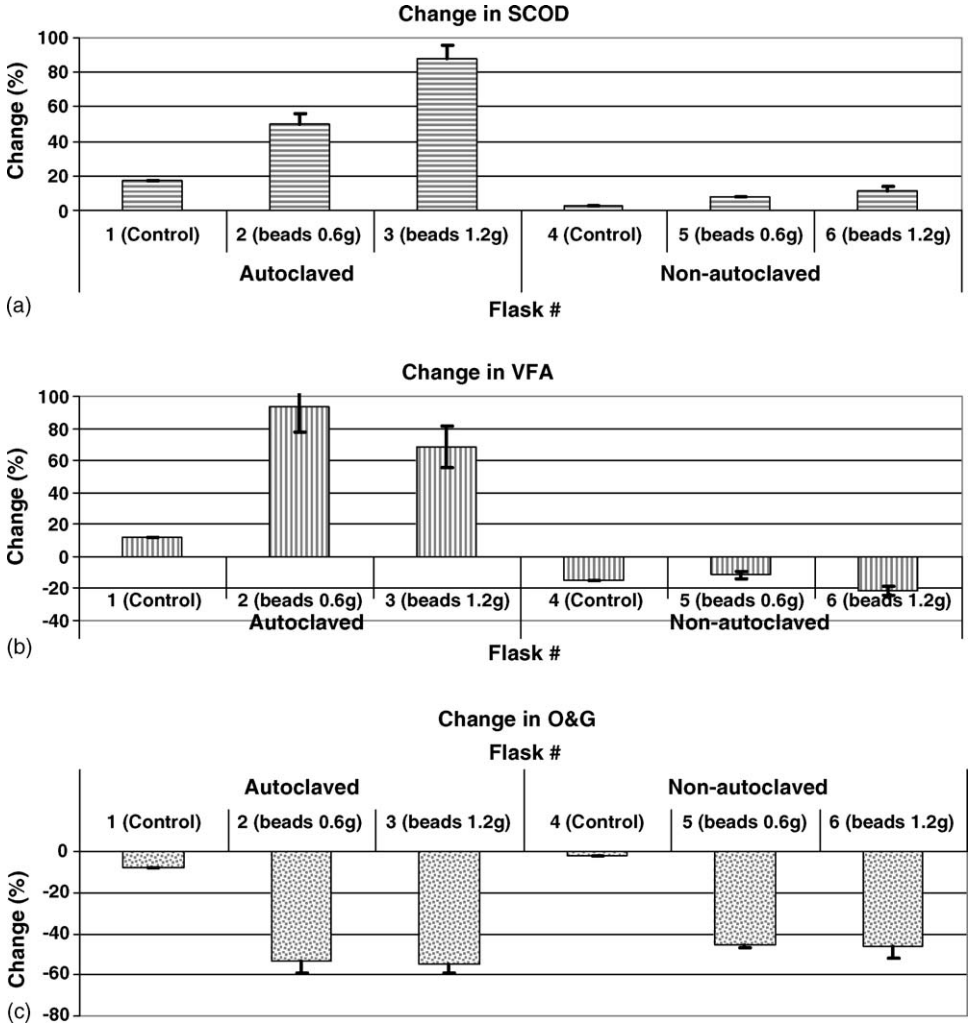

Fig. 6. Percentage change in (a) SCOD, (b) VFA and (c) O&G after 3 days in all the flasks.

Table 4
Percentage change in VFA components after 3 days in all the flasks

Flask#/VFA (%)[a]	1	2	3	4	5	6
Acetic acid	8	87 ± 7	60 ± 11	−27	47 ± 14	−46 ± 7
Propionic acid	17	138 ± 56	50 ± 22	−12	−16 ± 23	−5 ± 7
Isobutyric acid	119	56 ± 21	139 ± 53	44	109 ± 113	13 ± 5
Butyric acid	10	33 ± 2	74 ± 24	−30	38 ± 22	−10 ± 6
Valeric acid	−4	61 ± 30	55 ± 16	26	51 ± 35	13 ± 32
Isovaleric acid	−14	199 ± 50	195 ± 133	27	67 ± 4	30 ± 4

[a] 1–3: autoclaved, 4–6: non-autoclaved, 1&4: control, 2&5: 0.6 g beads, 3&6: 1.2 g beads.

Table 5
Mass balance based on COD

Flask #[a]	O&G as COD COD (g/L)		SCOD (mg/L)		VFA as COD (mg/L)		Mass as COD (g)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
1	14.04	12.91	3100	3620	723	733	8.19	8.04
2	14.04	6.51	3360	6050	706	1217	8.34 ± 0.06	6.50 ± 0.11
3	14.04	6.37	3085	5795	693	16 ± 0	8.16 ± 0.04	6.78 ± 0.21
4	14.04	13.76	3040	3120	742	764	8.17	8.11
5	14.04	7.71	3210	3450	737	804	8.27 ± 0.09	5.79 ± 0.03
6	14.04	7.60	3350	3735	742	775	8.35 ± 0.01	5.90 ± 0.41

[a] 1–3: autoclaved, 4–6: non-autoclaved, 1&4: control, 2&5: 0.6 g beads, 3&6: 1.2 g beads.

to microbial activity VFA decreased to 80–90% of the original by the 3rd day.

The individual components of free VFA are said to be the indicator of hydrolysis. In this experiment, it can be clearly seen that there was an increment in VFA components in enzyme treated samples (Table 4). Particularly, autoclaved samples were showing positive increments. The percentage increment in isovaleric acid in flask #2–3 was more than 100%. Autoclaved control sample (flask #1) was showing a slight increment in acetic acid (C_2), propionic acid (C_3), isobutyric acid, and butyric acid (C_4) but reduction in valeric acid (C_5) and isovaleric acid. Non-autoclaved control sample (flask #4) was showing the reverse change of flasks #1 where C_2–C_4 acids were reduced and C_5 was increased. This change can be due to microbial activity in the anaerobic environment. Flasks #2 was showing increment in all components during 3-day hydrolysis. Similar trends were achieved for flasks #3. It is clear that due to lipase hydrolysis, free fatty acids were released, hence increasing VFAs. However, there was not much difference in terms of VFA components at different dosages (0.6 and 1.2 g). Flasks #5 was showing reduction in most of the components during 3-day hydrolysis. However, reduction in VFA was less in flasks #6. From these results, it can be concluded that though due to lipase hydrolysis free fatty acids were released but due to microbial activities components were reduced.

The mass balance based on COD was calculated and shown in Table 5. Conservation of mass was achieved on the controls (#1 and 4) however, the lipase loaded samples were showing less mass after hydrolysis. This could be due to long chain fatty acids production, which was not measured in this experiment. Moreover, non-autoclaved samples (#5 and 6) showed lower final mass. This might be due to methane production under anaerobic condition, which was not accounted in the mass calculations.

After the hydrolysis experiment, the beads were filtered, washed and dried. About 60–80% of the beads were recovered (Table 6). The initial and final bead activities were measured

Table 6
Amount of beads used for hydrolysis and percentage recovery of beads and activity

Flask #[a]	Amount of beads			Activity			Total activity
	Initial (g)	Final (g)	Recovery (%)[b]	Initial (U/mg)	Final (U/mg)	Recovery (%)[c]	Recovered[d]
2	0.63 ± 0.01	0.39 ± 0.02	61 ± 2	760 ± 28	500 ± 28	66 ± 6	40 ± 2
3	1.23 ± 0.01	0.99 ± 0.02	81 ± 1	759 ± 4	531 ± 57	70 ± 7	57 ± 5
5	0.63 ± 0.01	0.39 ± 0.03	61 ± 6	757 ± 4	593 ± 24	78 ± 3	48 ± 6
6	1.20 ± 0.03	0.97 ± 0.04	80 ± 1	775 ± 18	565 ± 1	73 ± 1	59 ± 2

[a] 2–3: autoclaved, 5–6: non-autoclaved.
[b] Amounts of beads recovery = 100 × (initial weight−final weight)/initial weight.
[c] Recovery in activity = 100 × (initial activity−final activity)/initial activity.
[d] Total activity recovered = amounts of beads recovery × recovery in activity/100.

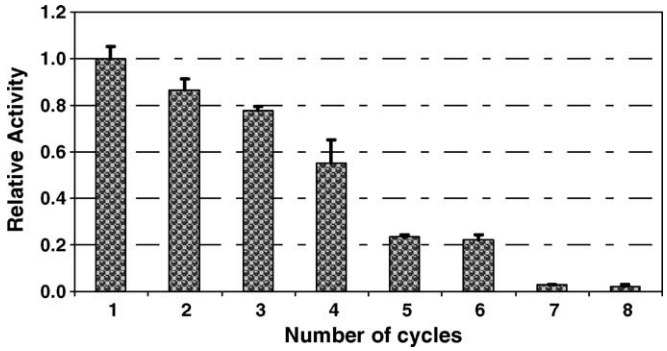

Fig. 7. Repeatability usage of immobilized lipase.

using lipase assay. About 60–80% activity remained in the beads. The total activity recovered (% bead weight recovered X% activity recovered) in this experiment was more than 50% and activity recovered increased with applied lipase dosage as expected. Moreover, the results of O&G consumed per unit mass of beads were showing a trend. In the autoclaved samples with 0.6 g of beads, about 12–15 g/L O&G was consumed per g of beads where as for the 1.2 g of beads only about 9–10 g/L O&G was consumed per g of beads. Similar trend was observed for the non-autoclaved samples. This might be due to complex nature of the reaction or due to product inhibition.

The repeatability test conducted at optimum conditions of beads and the results (Fig. 7) showed that the immobilized lipase could be used up to 4 cycles with a 55% activity recovery, which is comparable with literature [15]. However, after 4 cycles, the activity loss was critical. This is probably due to lipase leakage from the beads and/or blockage of substrate/product.

4. Conclusions

Immobilized lipase from *C. rugosa* could be used to hydrolyze fat in the pet food wastewater at a bead concentration of 0.5–1.5 g/L. Furthermore, the results showed that approximately 50% of the O&G was hydrolyzed due to enzyme activity. A significant increment in COD and VFA production was also observed. During the 3-day experiment, approximately 65% of the beads were recovered and after the hydrolysis, approximately 70% of the enzyme activity remained in the beads. This study shows the potential of immobilized lipase as a pre-treatment step in biological treatment of pet food manufacturing wastewater. Further studies should be conducted to optimize the experimental conditions and to evaluate the treatability through biological means.

Acknowledgement

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