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Hydrolytic pretreatment of oily wastewater by immobilized lipase

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Abstract

The purpose of this study was to evaluate the hydrolysis of wastewater with high oil and grease (O&G) concentration from a pet food industry using immobilized lipase (IL) as a pretreatment step for anaerobic treatment through batch and continuous-flow experiments. The intrinsic Michaelis constant (K_m) and maximum reaction rate (V_{max}) were estimated experimentally and the K_m value of IL (22.5 g O&G/L) was six-folds higher than that of the free lipase (FL) (3.6 g O&G/L), whereas V_{max} of both FL (31.3 mM/g min) and IL (33.1 mM/g min) were similar. Preliminary batch anaerobic respirometric experiments showed that chemical oxygen demand (COD) and O&G reduction were 49 and 45% without pretreatment and 65 and 64% with IL pretreatment respectively, while the maximum growth rate (μ_{max}) for pretreated wastewater (0.17 d^{-1}) was 3.4-folds higher than that of raw wastewater (0.05 d^{-1}) with similar Monod half-saturation constants ($K_s \sim 2.7$ g COD/L). The continuous-flow experimental study showed the feasibility of employing the hybrid packed bed reactor (PBR)-upflow anaerobic sludge blanket (UASB) system for the treatment of high-strength oily wastewater, as reflected by its ability to operate at an oil loading rate (LR) of 4.9 kg O&G/m^3 d (to the PBR) without any problems for a period of 100 days. During pseudo-steady-state conditions, the hybrid UASB produced relatively higher biogas compared to the control UASB, The effluent COD and O&G concentrations of hybrid system were 100 mg/L lower than that of the control UASB reactor and no foam production was observed in the hybrid UASB compared to the control UASB reactor.

Keywords: Immobilized lipase; Wastewater treatment; Hydrolysis; Oil and grease; Anaerobic processes

1. Introduction

Wastewaters from food processing industries have very high concentrations of oil and grease (O&G), solids and chemical oxygen demand (COD) levels, which are difficult to treat through conventional biological treatment system mainly due to slow biodegradability [1]. Anaerobic treatment processes are considered to be better than aerobic processes because of valuable biogas production, less biomass production, higher organic loading application and less energy consumption [2]. However, sludge flotation/washout [3,4] and O&G adsorption on the sludge surface [5,6] may reduce the treatment efficiency when treating oily wastewater. These problems could be overcome by enzymatic hydrolysis of O&G prior to anaerobic treatment [7].

Lipases (EC 3.1.1.3) are enzymes or biocatalysts, which have the ability to catalyze the hydrolysis of fats, oils, and grease (triacylglycerols) to free long-chain fatty acids (LCFAs) and glycerol. This hydrolysis reaction (forward direction) needs fat and an aqueous environment, which can be favorably achieved when using meat processing or pet food wastewater as a substrate. There are several research studies available on the treatment of oily wastes with artificially added fats using free lipase (FL) [7–12]. However, most of these studies focused on the pretreatment of wastewater with artificially added fats at low concentrations (<1 g/L) and there is very little information available on oily wastewater treatment using immobilized lipase (IL).

FLs are generally soluble and unstable, hence can be used only once in solutions. In addition, FL is not only often inactivated due to different environmental conditions (ionic strength, pH, inhibitors) but also too expensive to utilize in wastewater treatment. To overcome these problems, lipase can be immobilized on a suitable media. IL has the advantages of multiple usage, controlled reactions, and thermostability [13–15]. In addition, for continuous operation in packed bed reactors (PBR) or fluidized bed reactors, ILs yield higher dosage per unit volume of reactor and hence provide higher volumetric productivity compared to FLs [16]. Immobilization of enzymes can

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be achieved through matrix entrapment, which is based on the coupling of enzymes to the lattices of a polymer matrix [17]. Alginate is most widely used as matrix support because it is cheap, and has good gelling properties [15]. Moreover, alginate gel immobilization is a secure, fast, mild, and simple [18] procedure. However, high concentrations of sodium, potassium, phosphate, and chelating agents may destroy the beads [16,18] and the enzymes may leak out from the gel due to higher pore sizes of matrix [19]. Another entrapment method for lipase immobilization is the sol–gel method pioneered by Braun et al. [20], which was reported to achieve a high activity yield and increased stability [21]. However, this method yields brittle beads with diffusion limitations [22]. These problems may be overcome by synthesizing a composite alginate–silicate sol–gel matrix [23]. It has been proven that this co-immobilization technique can yield better stability upon ageing, limited enzyme leaching [24], better specific activity, and thermal stability compared to FL [22].

The purpose of this study was to evaluate the hydrolysis of high O&G containing pet food industrial wastewater using IL as a pretreatment to anaerobic treatment. Initially, the kinetic behavior of both enzymes (FL and IL) with oily wastewater as substrate was evaluated using Michaelis–Menten kinetic parameters. Subsequently, anaerobic batch respirometry tests were conducted for raw and IL pretreated wastewater to evaluate the impact of pretreatment on anaerobic biodegradability and the kinetic coefficients were estimated. Finally, the feasibility of IL pretreatment was further evaluated in a continuous-flow hybrid PBR-upflow anaerobic sludge blanket (UASB) reactor system to identify major operational issues and delineate cost and the performance comparison with a control UASB reactor system.

2. Materials and methods

2.1. Materials

Candida rugosa lipase (CRL) was obtained from Sigma-Aldrich Pvt. Ltd. (MO, USA) with an activity of 890 U/mg for hydrolysis of olive oil at 35 °C. Colloidal silica (LUDOX TM-50) and sodium alginate were also purchased from Sigma. Potassium silicate was kindly donated by PQ Corporation (PA, USA) and all the other chemicals were purchased from VWR International (Ont., Canada). All the chemicals used were reagent grade and used without further purification.

2.2. Analytical methods

Wastewater analysis was carried out in accordance with "standard methods" [25]. LCFA concentrations were analyzed using the method outlined in AOCS [26] and described in Jeganathan et al. [27].

2.3. Substrate

Oily wastewater from a local pet food industry was used as substrate in all the experiments. The characteristics of the raw wastewater are given in Table 1. Since the wastewater has very high-suspended solids and O&G, the feed was homogenized to obtain a uniform blend and diluted using tap water to 5% (v/v) strength for all the experiments.

Table 1
Raw wastewater characteristics

Parameter[a]	Value (38[b])	Method
Total chemical oxygen demand (TCOD)	54.9 ± 7.7	Hach
Soluble chemical oxygen demand (SCOD)	22.7 ± 3.7	Hach
Oil and grease (O&G)	22.5 ± 4.7	Gravimetric
Total suspended solids (TSS)	39.2 ± 4.0	Stantard methods
Volatile suspended solids (VSS)	20.2 ± 4.4	Stantard methods
Alkalinity	2.0 ± 0.7	Stantard methods
Free long-chain fatty acid (LCFA)	2.5 ± 0.9	GC
Volatile fatty acid (VFA)	14.9 ± 4.1	GC
pH	6.7 ± 0.3	pH meter

[a] All units except pH are in g/L.
[b] Number of samples.

2.4. Immobilization protocol

The CRL was immobilized on hybrid sol–gel/calcium alginate beads and the details are given in Trivedi et al. [23].

2.5. Enzyme assay

Lipase activities were determined by measuring the release of free fatty acids (FFA) by titration. Standard olive oil emulsion method [28] was used for olive oil (for activity confirmation only) and animal fat emulsion method [29] was used for pet food wastewater. Optimum assay conditions were employed for hydrolytic experiments with FL and IL. A pH of 7.2 and temperature of 35 °C was used as optimum conditions for FL as suggested by the supplier (Sigma-Aldrich Pvt. Ltd., MO, USA) while the pH was adjusted using 0.02 M Tris–HCl buffer. The optimum conditions for IL were found experimentally within the pH and temperature range of 4–9 and 25–40 °C to be at 6.8 and 37 °C, respectively. One unit of enzyme activity (U) was defined as the amount of enzyme, which liberated 1 μmol FFA/min under the assay conditions. Relative activity was defined as the ratio of activity at any condition to maximum activity of FL at optimum conditions.

2.6. Characterization of IL

The density, average mass, and average diameter were measured accurately. The particle size distribution was measured by using *Malvern* particle size analyzer (Mastersizer, Spectra Research Corporation, Ont., Canada). The average bead diameter and standard deviations were calculated from the data.

2.7. Batch treatability experiments

At first, the raw wastewater was pretreated with IL at optimum assay conditions prior to anaerobic treatability test. Diluted wastewater (5% v/v) was taken in 4–1 L flasks and IL beads were added at a rate of 0.04 g beads/g oil. The initial pH was adjusted with $NaHCO_3$ to 6.8 and kept in an orbital shaker at 150 rpm for

30 min at a temperature of 37 °C. Then the pretreated wastewater was left to settle for 10 min. Two distinct phases (supernatant and bottom sludge) were observed and the settled beads were removed from the flasks. A mixture of supernatant and bottom sludge (1:1, v/v) was used for anaerobic respirometry.

The respirometric experiments were conducted using 250 mL serum bottles capped with natural rubber sleeve stoppers (AER208 system, Challenge Environmental System, AR). Volumes of 70 mL of IL pretreated wastewater and 50 mL of seed sludge were filled in flasks. The acclimatized seed sludge used in this experiment was obtained from a laboratory-scale UASB reactor treating pet food wastewater [27]. Blanks were also prepared with raw wastewater and/or seed sludge. The pH of all samples was adjusted to neutral with $NaHCO_3$. The flasks were then kept in the temperature-controlled respirometer at 35 °C and mixed with magnetic stirrers. Each flask was connected to the bubble counter through a KOH trap, and methane gas production was recorded by a computer. The experiment was concluded when the biogas production stabilized. Initial and final samples were taken for analysis.

2.8. Continuous reactor system

The viability of IL pretreatment was evaluated in a continuous hybrid PBR-UASB reactor system and the performance was compared with a control UASB reactor system. The control UASB was made of PVC with a working volume of 15 L. The hybrid system was made of Plexiglas and comprised of a PBR (2 L) and a UASB (10 L). The schematic diagram of both systems is shown in Fig. 1 and the operating parameters are given in Table 2. The PBR of hybrid system was initially packed with 12 g of immobilized lipase beads and both the UASB reactors were seeded with anaerobic sludge from a full-scale anaerobic reactor treating ethanol wastewater. Both systems were operated at the same influent COD concentration for 100 days. Once the system attained pseudo-steady-state conditions on Day 65, the flow rates were doubled to evaluate the performance at higher organic loading rate (OLR) as seen from Table 2.

2.9. Hydrolysis rate

Hydrolysis experiments were carried out at the optimum assay conditions to quantify the amount of free LCFA production and the hydrolysis rate in terms of molecular LCFA production rate per unit mass of lipase, calculated in accordance with Eq. (1) for FL and IL.

$$\text{Hydrolysis rate} = \frac{\text{LCFA}_{\text{produced}}(\text{mg/L}) \times \text{Sample volume(mL)}}{\text{Molecular weight}_{\text{LCFA}}(\text{g/mol}) \times \text{Lipase(g)} \times \text{Time(min)}} \quad (1)$$

where, hydrolysis times for FL and IL were 10 and 30 min, respectively.

2.10. Kinetic parameters estimation

The lipase reaction rate (V) is related to the substrate concentration (S) by the Michaelis–Menten equation through a hyperbolic function with enzyme kinetic constants, that is, maxi-

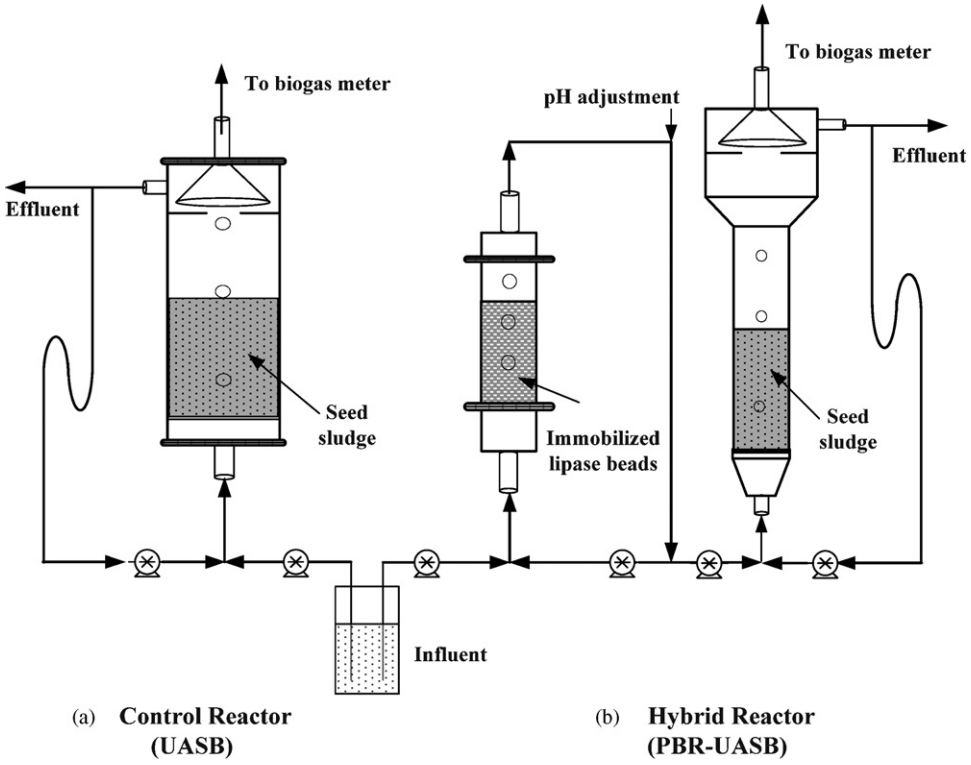

Fig. 1. Schematic diagram of the experimental setup.

Table 2
Operational parameters of control and hybrid systems

Parameter	Units	Control UASB		Hybrid PBR		Hybrid UASB	
		Phase I	Phase II	Phase I	Phase II	Phase I	Phase II
Duration	d	64	36	64	36	64	36
Flow Rate	L/d	6	12	4	8	4	8
HRT	d	2.5	1.25	0.5	0.25	2.5	1.25
OLR[a]	kg COD/m^3 d	1.3 ± 0.1	2.4 ± 0.2	6.5 ± 0.6	12.1 ± 0.8	1.3 ± 0.1	2.3 ± 0.1
Oil LR[b]	kg O&G/m^3 d	0.5 ± 0.1	1 ± 0.1	2.8 ± 0.5	4.9 ± 0.4	0.4 ± 0.1	0.6 ± 0.2

[a] Organic loading rate.
[b] O&G loading rate.

mum reaction rate (V_{max}) and intrinsic Michaelis constant (K_m). V_{max} is the maximum reaction rate attainable with the given amount of enzyme, when the enzyme is fully saturated with substrate. K_m is the substrate concentration, which gives half V_{max}, and K_m provides information about substrate binding to the enzyme. In this experiment these kinetic constants were estimated experimentally for the diluted oily wastewater at low oil concentrations (0.1–0.5 g O&G/L) using FL and IL. The data was analyzed using nonlinear regression fittings where the measured reaction rate (V_{meas}) was calculated by computing the instantaneous rate of FFA release and comparing it with the calculated reaction rate (V_{cal}) using the Michaelis–Menten equation until it matched.

Monod kinetic model was used for estimating the anaerobic kinetic coefficients. The initial values for the kinetic parameters, that is, maximum substrate removal rate (k) and half-saturation constants (K_s) were assumed within the range reported in the literature [30] and the theoretical methane production (cumulative) was calculated according to the initial values and compared with the respirometric results. The average percentage errors (APEs) between the measured and calculated values were minimized by changing the initial parameter values. The statistical significance was verified by comparing true average value with model value using APE. All the other data was analyzed using Excel 2003 with t-test (paired two samples for means) for parameters and models at 95% confidence intervals. All data reported are the average values from duplicate measurements.

3. Results and discussion

3.1. Immobilization of enzyme

The effect of ratio of sol–gel to alginate on retained activity was determined by varying alginate solution and lipase loading. The optimum ratio (v/v) was found to be at 1:2 (sol–gel: alginate) at a lipase loading of 20 g/L (data not shown), which yielded an enzyme activity equivalent to 80% of that of FL. The amount of lipase in the IL was found to be approximately 10% (by weight of the total beads). The protein content in the used hardening solution and washed deionized water was negligible. Therefore, almost complete immobilization could be attained. The density, average mass of bead, and average diameter were measured accurately for the immobilized beads. Replicate measurements showed that the bulk density was 650 ± 81 kg/m^3, solid density was 1150 ± 164 kg/m^3, average mass of a bead was about 2.53 ± 0.18 mg, and the average bead diameter (volume weighted average) was 82 μm.

3.2. Optimum ratio of lipase to substrate

Finding an optimum ratio of FL or IL to substrate is vital due to economic reasons. In this case, different amounts (0–0.1 g) of FL and IL were added to the diluted oily wastewater (5% strength, v/v) and the release of free acids was measured at optimum assay conditions. Results (data not shown) showed that the optimum amounts of FL and IL for hydrolyzing 1 g of O&G were about 0.02 and 0.04 g, respectively. Since the IL beads contained about 10% of FL, only about 0.004 g FL is necessary for the hydrolysis by IL as opposed to 0.02 g FL.

3.3. Effect of hydrolysis on LCFA and soluble chemical oxygen demand (SCOD)

In general, lipase hydrolysis of fat produces free LCFAs and water-soluble glycerol. Several hydrolysis experiments (for both FL and IL) were carried out at the optimum assay conditions to quantify the amount of free LCFA production and the results are shown in Fig. 2. As seen from Fig. 2, the total free LCFA increments were about 155 and 85% for FL and IL, respectively. Lower LCFA increment in IL is attributed to mass transfer limitations. The hydrolysis rate in terms of molecular LCFA production rate per unit mass of lipase was calculated in accordance with Eq. (1) and given in Table 3. Although the rates for palmitic and oleic acids were comparable for both FL and IL, the rates for the other LCFAs were 20–40% lower

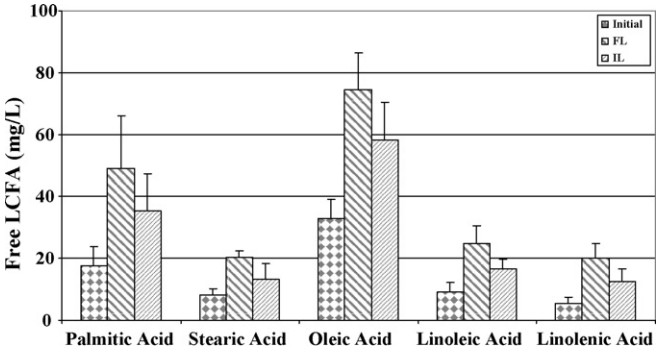

Fig. 2. Free LCFA formation during hydrolysis.

Table 3
Individual LCFA hydrolysis rates

LCFA	MW (g/mol)	Initial LCFA (mg/L)	LCFA increment		Hydrolysis rate	
			FL (mg/L)	IL (mg/L)	FL (μmol/g min)	IL (μmol/g min)
Palmitic acid	256	18 ± 6	31.3	17.7	15.3	14.4
Stearic acid	284	8 ± 2	12.0	5.0	5.3	3.7
Oleic acid	282	33 ± 6	41.3	25.3	18.3	18.7
Linoleic acid	280	9 ± 3	15.3	7.4	6.8	5.5
Linolenic acid	278	5 ± 2	14.7	7.2	6.6	5.4

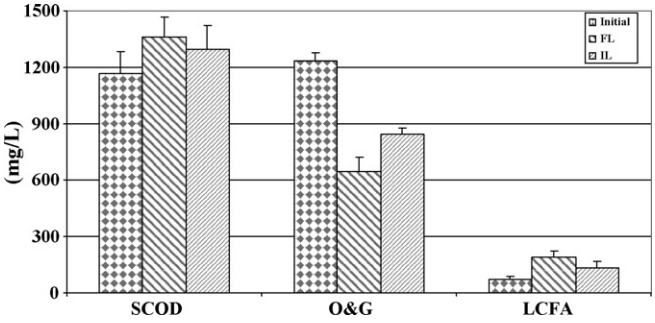

Fig. 3. Change in SCOD, O&G, and free LCFA (as COD).

in the case of IL as compared to FL. It is interesting to note that the substrate (pet food wastewater) used in this experiment contained 50% oleic acid and 27% palmitic acid and all the other LCFAs were less than 10% [27], hence the free LCFA production rates were comparable for FL and IL. Furthermore, as shown in Fig. 3, the reduction in O&G was about 48 and 32% and the increase in SCOD was about 17 and 11% for FL and IL, respectively. Hence, the results of the preliminary batch experiments confirmed that both the FL and IL behaved similarly and the prehydrolysis of wastewater with high O&G by lipase could be beneficial as pretreatment for anaerobic processes.

3.4. Estimation of enzyme kinetic constants

The enzyme kinetic constants V_{max} and K_m for both FL and ILs are summarized in Table 4. The experiments were conducted at the optimum assay conditions and the values for IL were reported as apparent values (i.e. reflecting mass transfer limitations) as opposed to true values. As seen from Table 4, V_{max} values were similar for both FL (31.3 mM/g min) and IL (33.1 mM/g min); however, the K_m value of IL (22.5 g oil/L) was found to be six-folds higher than that of the FL (3.6 g oil/L). Similar results were obtained by Pereira et al. [28] where, CRL was immobilized on chitosan and olive oil was used as substrate. From their study, the V_{max} values were about 38 and 51 mM/g min for FL and IL, respectively and the K_m value of IL (0.42 M) was three-folds higher than that of FL. The comparability of V_{max} for the FL and IL suggests that there are no major structural changes in the enzyme during the immobilization process. In a continuous-flow system, a lower K_m value implies that the enzymatic reaction follows first order kinetics, where the reaction rate increases with the increase in influent O&G concentration. K_m also influences the effluent O&G concentration as the effluent concentration increases with the increase in K_m. However, variations in influent O&G concentrations will more strongly impact the effluent O&G concentrations at lower K_m values than at high K_m because the system is operating at maximum reaction rate. Alternatively, with high K_m, the reaction rate will increase with increase in influent O&G concentration but the effluent O&G will not increase proportionately. From the batch kinetic studies, K_m for IL was higher than FL, which might be due to mass transfer limitations [31], thus implying that the diffusion of O&G through the packing is the rate-limiting step. As seen from Fig. 3, both O&G hydrolysis and free LCFA production were relatively higher in FL compared to IL. In the case of continuous-flow systems, the resistance to the diffusion of substrate into the immobilized enzyme is expected to be similar to or even more than the batch experiments, which would translate to higher O&G concentrations in effluent, as compared with the use of FL for hydrolysis.

Table 4
Kinetic constants of FL and IL for oily wastewater

Parameter	FL	IL[a]	Units
Michaelis constant (K_m)	3.57	22.48	g O&G/L
Maximum reaction rate (V_{max})	31.30	33.10	mM/g min
Average percentage error[b] (APE)	2.6	4.3	%

[a] Apparent values.

[b] APE = Average Percentage error = $\sum_{1}^{n} \frac{|V_{cal} - V_{meas}|/V_{meas}}{n}$ where, V is reaction rate, n is number of samples.

3.5. Repeated use of IL

The repeatability test was conducted at optimum conditions and the results (Fig. 4) show that the IL could be used up to four cycles, each lasting for 30 min with a retained activity of 55%. However, after four cycles, the activity loss is critical. This is probably due to lipase leakage from the beads and/or blockage by substrate/product. Accordingly, for continuous (four cycles) hydrolysis of oily wastewater, about 53 mg of IL (which contains ca. 5 mg of FL) is sufficient for 1 g of O&G hydrolysis compared to 20 mg of FL.

3.6. Batch treatability studies

Preliminary respirometric studies were performed to evaluate the effect of IL pretreatment on the anaerobic process. Samples

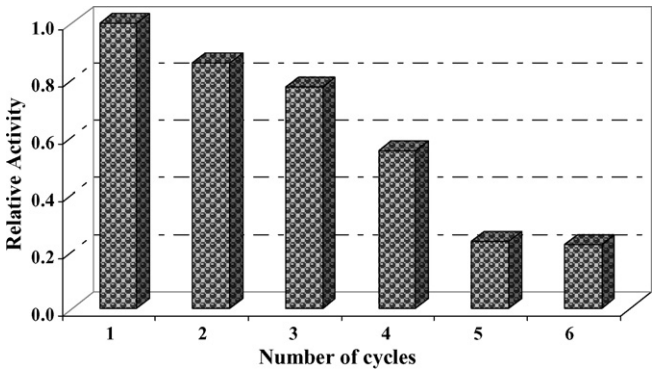

Fig. 4. Repeatability usage of IL.

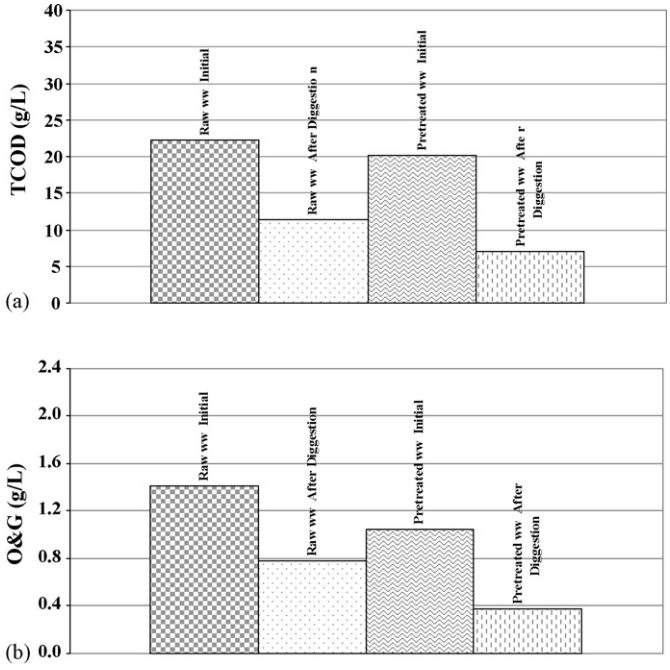

Fig. 5. (a) Variation in TCOD in respirometric studies. (b) Variation in O&G in respirometric studies.

were taken for analysis before and after the anaerobic respirometric studies. As seen from Fig. 5, the results showed that total chemical oxygen demand (TCOD) and O&G reductions were about 49 and 45% without pretreatment and 65 and 64% with pretreatment, respectively. Since the pretreated wastewater used in the respirometry was a mixture of the supernatant and bottom sludge (1:1, v/v), the TCOD was slightly lower than that of raw wastewater. Kinetic (Monod) parameters were estimated from methane gas production and initial values of COD and volatile suspended solids (VSS) (Table 5) for raw and lipase pretreated wastewater. The maximum growth rate (μ_{max}) for pretreated wastewater ($0.17\,d^{-1}$) was 3.4-folds higher than that of raw wastewater ($0.05\,d^{-1}$) with similar Monod half-saturation constants ($K_s \sim 2.7$ g COD/L). This clearly shows that the IL pretreatment enhanced anaerobic treatability.

3.7. Continuous system performance

The purpose of the PBR was to hydrolyze the O&G to free LCFAs and therefore, the TCOD reduction across the PBR was negligible. Consequently, both the control UASB and hybrid UASB reactor operated at the same OLR and hydraulic retention time (HRT) throughout the study as given in Table 2. The oil loading rate (LR) was reduced to hybrid UASB compared to control UASB due to O&G hydrolysis in PBR. As seen from Table 2, the control UASB reactor was fed at an oil LR of 0.5 ± 0.1 and 1 ± 0.1 kg O&G/m^3 d whereas the hybrid reactor was fed at 0.4 ± 0.1 and 0.6 ± 0.2 kg O&G/m^3 d for phases I and II, respectively. Fig. 6a shows the temporal variations of oil LR for both the control and hybrid UASB reactors while Fig. 6b depicts the variation of influent and effluent COD. Similarly, Fig. 6c shows the variation of O&G concentrations in both reactors. As seen from Fig. 6b, the effluent COD of control UASB reactor was about 400 and 450 mg/L in phases I and II, respectively as compared to 285 and 200 mg/L for hybrid UASB reactor. Furthermore, as seen from Fig. 6c, the effluent O&G was about 170 and 150 mg/L for the control UASB reactor and 65 and 60 mg/L for the hybrid UASB reactor in phases I and II, respectively. From these results, it is evident that the effluent COD and O&G concentrations of hybrid system were not only 40% lower than that of control UASB reactor, but also varied less widely. Several processes such as adsorption on the seed sludge, hydrolysis, biodegradation, and desorption influence the fate of O&G in the UASB reactor. In the control UASB reactor, without enzyme, the hydrolysis rate is expected to be very slow relative to adsorption and desorption. Thus, the cyclical

Table 5
Anaerobic kinetic coefficients

Parameter	Literature range[a]	Raw ww[b]	Pretreated ww[b]
Yield coefficient, Y_0 (g VSS/g COD)	0.04–0.17	0.05	0.08
Intrinsic max substrate removal rate, k (d^{-1})	0.77–6.67	0.96	2.16
Maximum growth rate, μ_{max} (d^{-1})	0.08–0.55	0.05	0.17
Intrinsic half-saturation coefficient, K_s (g COD/L)	0.11–3.18	2.67	2.80
Decay rate, k_d (d^{-1})	0.01–0.015	0.015	0.011
Initial COD concentration (mg COD/L)		1705	1407
Initial total biomass (g VSS/L)		16.2	16.4
Average percentage error (APE) (Cum. Gas)		13	28
Average percentage error (APE) (Gas Prod. Rate)		15	8

[a] [30].
[b] Wastewater.

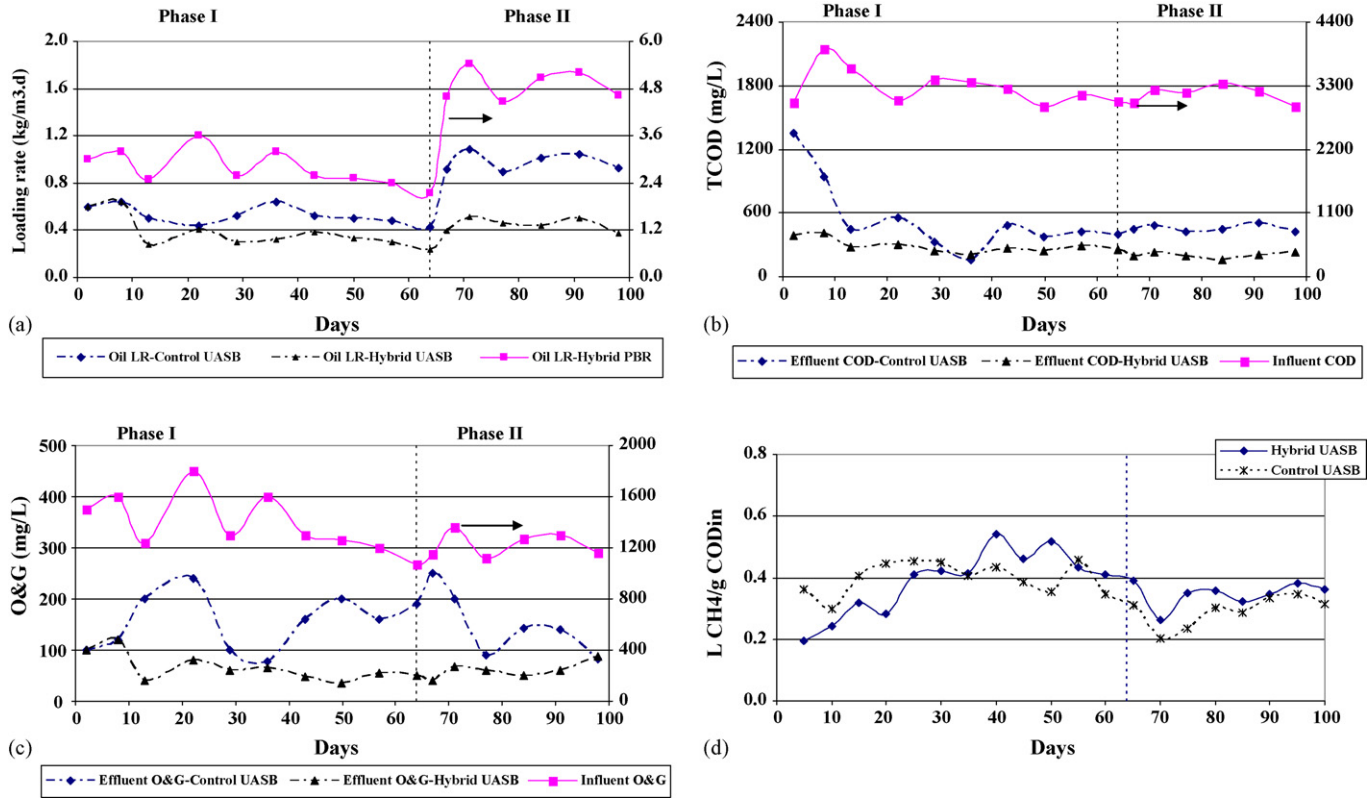

Fig. 6. (a) O&G loading rates for control and hybrid systems. (b) Influent and effluent COD of control and hybrid systems. (c) Influent and effluent O&G of control and hybrid systems. (d) Average methane production per influent COD.

trend of the effluent O&G in the control UASB (Fig. 6c), which did not match the influent O&G variations can be attributed to the predominant impact of sorption and desorption. In the test UASB, due to the prehydrolysis of O&G to free LCFAs in the PBR, the rates of adsorption, biodegradation, and desorption are anticipated to be much higher than the control, thus stabilizing the effluent quality. Furthermore, the hybrid UASB received a uniform O&G due to hydrolysis in PBR. In this study, diurnal biogas production rate was measured using a wet tip gas meter (Rebel point Wet Tip Gas Meter Co., Nashville, TN) and the methane content was measured regularly at about 68–70% in both UASB reactors. Fig. 6d illustrates the temporal variation of methane production per unit influent COD. During the pseudo-steady state period, the control UASB produced 0.39 ± 0.08 and 0.29 ± 0.06 L CH_4/g COD_{in} whereas the hybrid UASB produced 0.46 ± 0.08 and 0.34 ± 0.05 L CH_4/g COD_{in} in phases I and II, respectively. It must be asserted that the differences in methane production per influent COD between the control and hybrid UASB were found to be statistically significant at the 95% confidence level.

Generally, anaerobic treatment of high O&G wastewater is hindered by foam or sludge flotation [32]. In this comparative study, the control UASB reactor produced about 0.18% ($v_{foam}/v_{influent}$) foam on Day 51 which was removed manually from the top of the reactor every week at a rate of 0.12 L/week. Initially, there was no foam production in the control reactor due to the adsorption and accumulation of O&G onto the biomass in the bed. With time, the accumulation increased which caused the effective density of biomass to decrease below that of water, thus leading to foam production. The analysis of foam showed that the foam contained TCOD of 168 ± 125 g/L and O&G of 58 ± 44 g/L. The foam also contained lighter seed sludge and hence, this reduced the amount of seed sludge in the bed. On the other hand, the hybrid UASB reactor did not produce any foam till Day 100 due to prehydrolysis in PBR, which reduced the oil LR to the hybrid UASB (Table 2).

The performance of the PBR was hampered occasionally by the instability of IL beads, which broke into pieces especially in phase II. Consequently, the broken beads were removed and the inner wall of the reactor was cleaned to remove attached O&G every week. The O&G in the cleaning water was only about 0.06% (w/w) of the influent O&G. They were replaced with fresh beads at the rate of 4.6 and 12.5 g/week for phases I and II, respectively. Strong support media such as glass beads could be used to immobilize the lipase to reduce the replacement frequency.

Overall cumulative COD mass balances were carried out for both UASBs at different phases. The influent COD was equated to the summation of COD effluent, sludge accumulation, foam, and biodegradation using the weekly data and the calculation details are given in Jeganathan et al. [27]. The COD was generally balanced within 10%. As seen from Table 6, about 82 and 89% of the influent COD was removed by biodegradation in phase I in the control and hybrid UASBs, respectively. How-

Table 6
COD balance (as % of influent COD)

COD (%)	Phase I		Phase II	
	Control	Hybrid	Control	Hybrid
Effluent	10.2	8.4	14.1	6.3
Sludge	1.2	2.5	2.1	2.3
Foam	4.0	0.0	7.7	0.0
Degraded	81.7	88.9	73.3	81.8
Unaccounted	3.0	0.2	2.7	9.6

ever, the increase in OLR in phase II caused an increase in foam, which reduced the degradation in the control UASB whereas hybrid UASB reactor was unaffected.

3.8. Commercial viability

In this research, purified lipase at $\$1\,g^{-1}$ (Sigma-Aldrich Pvt. Ltd, MO, USA) was used to evaluate the treatability of oily wastewater. From the continuous experiment with hybrid reactor system, the cost of the treatment was calculated to be about $\$100\,m^{-3}$ of wastewater based on purified lipase costs. Nevertheless, the cost of treatment could be reduced to as low as $\$2\,m^{-3}$ based on the cost of commercial lipase ($\$4\,kg^{-1}$) with 20% of the activity of purified lipase. Hence, the use of unpurified, commercial lipase makes the hydrolytic pretreatment technology more cost-effective.

4. Conclusions

From the study on the hydrolytic pretreatment of oily wastewater by the IL, the following conclusions could be drawn:

1. The K_m value of IL (22.5 g O&G/L) was six-folds higher than that of the FL (3.6 g O&G/L) where, V_{max} of both FL (31.3 mM/g min) and IL (33.1 mM/g min) were similar.
2. The repeatability test showed that the IL could be used up to four cycles with a retained activity of 55%.
3. Preliminary anaerobic respirometric experiments confirmed the biodegradability of wastewater pretreated by IL and the COD and O&G reduction were 49 and 45% without pretreatment and 65 and 64% with pretreatment, respectively, while the maximum growth rate of pretreated wastewater ($0.17\,d^{-1}$) was 3.4-folds higher than that of raw wastewater ($0.05\,d^{-1}$) with similar Monod half-saturation constants ($\sim$2.7 g/L).
4. The continuous-flow experimental study showed the potential advantages of the hybrid PBR-UASB system over the control system for the treatment of high-strength oily wastewater where the hybrid system was operated up to an oil LR of 4.9 kg O&G/m^3 d (to the PBR) without any problems for a period of 100 days. The hybrid system produced nearly 18% higher gas than the control system and 100 mg/L lower effluent COD and O&G concentrations. Moreover, no foam production was observed in the hybrid UASB compared to the control UASB reactor ($0.18 \pm 0.14\%\ v_{foam}/v_{wastewater}$).

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References

[1] G. Nakhla, M. Al-Sabawi, A. Bassi, V. Liu, Anaerobic treatability of high oil and grease rendering wastewater, J. Hazard. Mater. 102 (2003) 243–255.
[2] G. Lettinga, Sustainable integrated biological wastewater treatment, Water Sci. Technol. 33 (3) (1996) 85–98.
[3] C.S. Hwu, S.K. Tseng, C.Y. Yuan, Z. Kulik, G. Lettinga, Biosorption of long-chain fatty acids in UASB treatment process, Water Res. 32 (1998) 1571–1579.
[4] C.-S. Hwu, B. Donlon, G. Lettinga, Comparative toxicity of long-chain fatty acid to anaerobic sludges from various origins, Water Sci. Technol. 34 (5/6) (1996) 351–358.
[5] A. Rinzema, M. Boone, K. van Knippenberg, G. Lettinga, Bactericidal effect of long chain fatty acids in anaerobic digestion, Water Environ. Res. 66 (1) (1994) 40–49.
[6] M.A. Pereira, A.J. Cavaleiro, M. Mota, M.M. Alves, Accumulation of long chain fatty acids onto anaerobic sludge under steady state and shock loading conditions: effect on acetogenic and methanogenic activity, Water Sci. Technol. 48 (6) (2003) 33–40.
[7] M.C. Cammarota, G.A. Teixeira, D.M.G. Freire, Enzymatic pre-hydrolysis and anaerobic degradation of wastewaters with high fat contents, Biotechnol. Lett. 23 (2001) 1591–1595.
[8] L. Masse, K.J. Kennedy, S.P. Chou, The effect of an enzymatic pretreatment on the hydrolysis and size reduction of fat particles in slaughterhouse wastewater, J. Chem. Technol. Biotechnol. 76 (2001) 629–635.
[9] L. Masse, D.I. Masse, K.J. Kennedy, Effect of hydrolysis pretreatment on fat degradation during anaerobic digestion of slaughterhouse wastewater, Process Biochem. 38 (2003) 1365–1372.
[10] M. Murray, D. Rooney, V.M. Neikerk, A. Montenefro, L.R. Weatherley, Immobilization of lipase onto lipophilic polymer particles and application to oil hydrolysis, Process Biochem. 32 (1997) 479–486.
[11] W. Mongkolthanaruk, S. Dharmsthiti, Biodegradation of lipid-rich wastewater by a mixed bacterial consortium, Int. Biodeterior. Biodegrad. 50 (2002) 101–105.
[12] A.B. German, A.D. Neklyudov, A.N. Ivankin, A.V. Berdutina, The kinetics of hydrolysis of animal fat by *Pancreatic* lipase, Appl. Biochem. Microbiol. 38 (6) (2002) 517–520.
[13] M. Matsumoto, K. Ohashi, Effect of immobilization on thermostability of lipase from *Candida rugosa*, Biochem. Eng. J. 14 (2003) 75–77.
[14] C. Chiou, W. Wu, Immobilization of *Candida rugosa* lipase on chitosan with activation of the hydroxyl groups, Biomaterials 25 (2004) 197–204.
[15] Z. Knezevic, S. Bobic, A. Miutinovic, B. Obradovic, L. Mojovic, B. Bugarski, Alginate-immobilized lipase by electrostatic extrusion for the purpose of palm oil hydrolysis in lecithin/isooctane system, Process Biochem. 38 (2002) 313–318.
[16] W. Hartmeier, Immobilized Biocatalysts—An Introduction, Springer-Verlag, Berlin, Germany, 1988.
[17] S. Benjamin, A. Pandey, Review: Candida rugosa lipases: molecular biology and versatility in biotechnology, Yeast 14 (1998) 1069–1087.
[18] P. Germeiner, Enzyme Engineering—Immobilized Biosystems, Ellis Horwood, West Sussex, 1992.
[19] H. Tanaka, M. Matsumura, I.A. Veliky, Diffusion characteristics of substrates in Ca-alginate gel beads, Biotechnol. Bioeng. 26 (1984) 53–58.
[20] S. Braun, S. Rappoport, R. Zusman, D. Avnir, M. Ottolenghi, Biochemically active sol–gel glasses: the trapping of enzymes, Mater. Lett. 10 (1990) 1–8.
[21] G. Kuncova, M. Sivel, Lipase immobilized in organic–inorganic matrices, J. Sol–Gel Sci. Technol. 8 (1997) 667–671.
[22] J. Chen, H. Wang, Improved properties of bilirubin oxidase by entrapment in alginate-silicate sol–gel matrix, Biotechnol. Tech. 12 (1998) 851–853.

[23] U.J. Trivedi, A. Bassi, J. Zhu, Investigation of phenol removal using sol–gel/alginate immobilized soybean seed hull peroxidase, Can. J. Chem. Eng. 84 (2006) 239–247.
[24] T. Coradin, C. Livage, Mesoporous alginate/silica biocomposites for enzyme immobilization, Chimie 6 (2003) 147–152.
[25] APHA, ASCE and WEF. Standard Methods for the Examination of Water and Wastewater, 20th ed. Washington DC, USA, 1998.
[26] AOCS, Official methods and recommended practices of the AOCS, Method Ce 2-66 Champaign, IL, American Oil Chemists' Society, 1998.
[27] J. Jeganathan, G. Nakhla, A. Bassi, Long-term performance of high-rate anaerobic reactors for the treatment of oily wastewater, Environ. Sci. Technol. 40 (2006) 6466–6472.
[28] E.B. Pereira, H.F. de Castro, F.F. de Moraes, G.M. Zanin, Kinetic studies of lipase from *Candida rugosa*—A comparative study between free and immobilized enzyme onto porous chitosan beads, Appl. Biochem. Biotechnol. 91 (2001) 739–752.
[29] M.D. Virto, I. Agud, S. Montero, A. Blanco, R. Solozabal, J.M. Lascaray, M.J. Llama, J.L. Serra, L.C. Landeta, M. de Renobales, Hydrolysis of animal fats by immobilized *Candida rugosa* lipase, Enzyme Microb. Technol. 16 (1994) 61–65.
[30] S.G. Pavlostathis, E. Giraldo-Gomez, Kinetics of anaerobic treatment, Water Sci. Technol. 24 (1991) 35–59.
[31] J. Shaw, R. Chang, Lipolytic activities of a lipase immobilized on six selected supporting materials, Biotechol. Bioeng. 35 (1990) 132–137.
[32] G. Vidal, A. Carvalho, R. Mendez, J.M. Lema, Influence of the content in fats and proteins on the anaerobic biodegradability of dairy wastewaters, Bioresour. Technol. 74 (2000) 231–239.