Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2013.871582

53 (2015) 2450–2455
March

Taylor & Francis
Taylor & Francis Group

Pre-treatment of high fat content dairy wastewater using different commercial lipases

Tejal V. Adulkar, Virendra K. Rathod*

Department of Chemical Engineering, Institute of Chemical Technology, Matunga (E), Mumbai 400019, India
Tel. +91 22 33612020; Fax: +91 22 33611020; email: vk.rathod@ictmumbai.edu.in

Received 29 July 2013; Accepted 30 October 2013

ABSTRACT

To overcome the shortcomings of direct anaerobic digestion of the dairy waste effluent, an enzymatic pre-treatment have been employed to significantly overcome the sludge problems and other drawbacks. This work describes the application of Lipase *Z* to perform the enzymatic pre-hydrolysis of a synthetic dairy wastewater containing around 2,000 mg/mL of fat content. Different process parameters like effect of enzyme loading, temperature, different concentration of fats and different concentrations of NaCl were optimized for maximum conversion of fat. The maximum hydrolysis of around 75% is achieved at 0.2% w/v enzyme loading, 30°C and 100 mM NaCl concentration for 2,000 mg/mL fats content. It was contemplated that the enzymatic pre-hydrolysis prior to the anaerobic digestion reduced the reaction time required for anaerobic degradation, improved biogas formation and appreciative chemical oxygen demand removal.

Keywords: Dairy wastewater; Anaerobic treatment; Pre-treatment; Enzymes; Lipase

1. Introduction

Dairy industry is one of the major industries in India with around 15% growth rate and is estimated to cross 150 million tonnes per annum production [1]. Lately, the increase in production of milk and milk products has also led to immense advancement of dairy industries all over the world [2]. Thereupon, the magnitude of effluent being generated and discharged from these industries is also increased. Thus, the dairy industry is one of the prime pollution causing industries and pollution potential of this leading economic sector cannot be neglected. The source of dairy wastewater is basically from various steps in dairy chain of cleaning and washing equipments in the plant production processing, packaging transportation, storage and residual milk [2–4]. These dairy wastewaters are highly concentrated with organic molecules like proteins, carbohydrates, lipids and also contain large amount of suspended solids, BOD, chemical oxygen demand (COD), oil and grease content. In addition, it is also composed of detergent and sanitizer. All these aspects adding up to the complexity of wastewater treatment. The organic content in dairy wastewater is a major factor affecting the biodegradability coefficient of dairy wastewater and induces serious problems like gross land and water pollution, bad environmental impact and organic load on municipal sewage system [3,5]. Oils and grease being

*Corresponding author.

slow degrading compounds cause problems in the aeration and pumping system. On the other hand, the formation of greasy layer on the surface of aeration tanks interferes with the sedimentation and flocculation of biomass and hinders the gas transfer required for the biological degradation [6]. Also, it exhausts the dissolved oxygen levels of the receiving streams and moreover generating foul odour creating nuisance conditions. If oil and greases are discharged without treatment, it can induce severe environmental pollution by forming oily film on the water surface, preventing oxygen diffusion and in turn hampering the aquatic life. Thus, it is necessary to generate effective technique to lessen the negative environmental impact of dairy wastewater [7,8].

The available physical and chemical treatment methods are fast and effective but suffer from various drawbacks such as requirement of costly reagent, harsh reaction conditions, hazardous reagents and higher energy consumption. Therefore, the biological processes are favoured [3]. In biological methods, the anaerobic treatment process is preferred over the aerobic process due to several advantages like valuable biogas production, no oxygen required and therefore less energy consumption [1,3]. However, excessive fats, oils and greases (FOGs) showed inhibitory action on the anaerobic degradation due to accumulation of the greasy scum layers limiting the gas transfer and further mixing [6]. Thus, this excessive FOG leading to process failure was needed to be controlled by development of a pre-treatment process to accelerate the anaerobic digestion and increasing the degree of stabilization in comparison to classical anaerobic digestion without a pre-treatment process [1,9].

Amongst all the alternatives, the enzymatic approach acquired more considerable attention because of the stringent environmental regulations due to the clean and eco-friendly enzyme applications [10]. Eminently, they can be operated under milder conditions with respect to pH and temperature. Thus, the enzymes are more satisfying due to their biodegradability and emerging as a green alternative for other treatment processes [11].

Lipases (E.C.3.1.1.3) fall in to the hydrolases category catalysing the lipid/oil hydrolysis to long-chain fatty acids and glycerol [12]. These enzymes act on the lipid water interface and particularly hydrolyse the O & Gs which usually causes clogging and other severe problems like fat scum layer at the surface, granular mass flotation, etc. in the upflow anaerobic sludge blanket reactor [10,13]. Hence, they are of much interest in high fat content industries like dairy industries. Also, there is literature available on the use of enzyme for dairy wastewater pre-treatment [5,7,10].

The objectives of this work are (i) Screening and utilization of lipase enzymes for dairy wastewater pre-treatment. (ii) To optimize the reaction parameters for maximum FOG conversion. (iii) To analyse the efficiency of the pre-treatment by its reduction in COD and FOG content.

2. Materials and methods

2.1. Materials

2.1.1. Enzymes

Lipase *Z* (lipase from *Candida rugosa*-free form) was obtained as a gift sample from Zytex, Mumbai. It has an activity of 360,000 units per gm.

2.1.2. Wastewater

Dairy wastewater was prepared synthetically by diluting different proportions of milk in deionized water to get various fat concentrations. The amount of fat content was prepared approximately in the range 2,000, 4,000, 6,000 and 8,000 mg/mL higher than the normal fat content of dairy wastewater which ranges from 200 to 1,200 mg/mL. The milk was bought from a local dairy, Sharma Dairy, Mumbai. The milk:water ratio of 1:8 was used for the optimization study (2,000 mg/mL). This wastewater prepared was used for the pre-hydrolysis with the help of enzymes and for studying the characteristics of dairy wastewater. When not in use, prepared waste water was stored in deep freezer.

All solvents and reagents are of analytical grade and purchased from S.D. Fine Chemical, Mumbai, India.

2.2. Analytical methods

2.2.1. COD

It was determined by oxidation of the small amount of sample with potassium dichromate and sulphuric acid (Standard Methods of Water and Wastewater Examination, APHA, 1995).

2.2.2. Fats, oils and greases

FOGs were determined by the extraction of wastewater in a reflux using hexane as a solvent as described in the Standard Methods of Water and Wastewater Examination, APHA, 1995.

The acidity of the raw wastewater was estimated by titrating it with 0.1 N NaOH solution to neutralize the free fatty acids levels. The characteristics of the dairy wastewater used for the study has been stated in Table 1.

2.2.3 Titrimetric analysis

The free fatty acids formed in the hydrolysis reaction were evaluated by titrating it with 0.02 N aqueous KOH solution. The free fatty acid formation was used to supervise the reaction progress.

2.3. Experimental method

In a baffled glass reactor (150 mL capacity) of 4.5 cm i.d. equipped with a six-bladed turbine glass impeller, 100 mL of crude wastewater was fed along with an enzyme (0.2% w/v) and sodium chloride (100 mM) as an emulsifying aid. The entire reactor assembly was immersed in a thermostatic water bath and maintained at required temperature with an accuracy of ± 5°C. The temperature of the reaction was being monitored with the help of a temperature controller. The agitation was provided by means of an electric motor equipped with speed controlling device. The reactions were carried out for a maximum period of 24 h and at 200 rpm. At regular intervals, a small sample (2 mL) was withdrawn from the reactor and transferred to conical flask. Ten millilitres of a 50:50 v/v mixture of acetone in ethanol were added to the sample to denature the enzyme and freeze the reaction. This mixture was titrated with standard 0.02 M potassium hydroxide (KOH) solution using phenolphthalein as an indicator to find the acid value and percentage conversion.

3. Results and discusssion

3.1. Screening of lipases

Lipases from different microbial strains were screened based on their capability to hydrolyse the high molecular FOGs in the dairy wastewater in to fatty acids and glycerol. Four different commercially available lipases namely, *Novozym 435*, *Lipozyme RM IM* (immobilized), *Pseudomonas fluorescens* and *Candida rugosa* lipase (free) were screened to obtain maximum conversion of triglycerides (fats) in to fatty acid. The pH of the crude wastewater was found to be around 6.5. Fig. 1 shows that amongst all *Candida rugosa* lipase showed satisfying results, and hence was chosen for the further enzymatic studies.

Table 1
Characteristics of the dairy wastewater used in this study

Parameters	Value
pH	6–6.5
Density (g/cm^3)	1.005
Free fatty acids (%)	0.28
Lipids/FOG (mg/L)	2,000
COD (ppm)	42,000
Acidity (mg.NaOH/L)	0.4

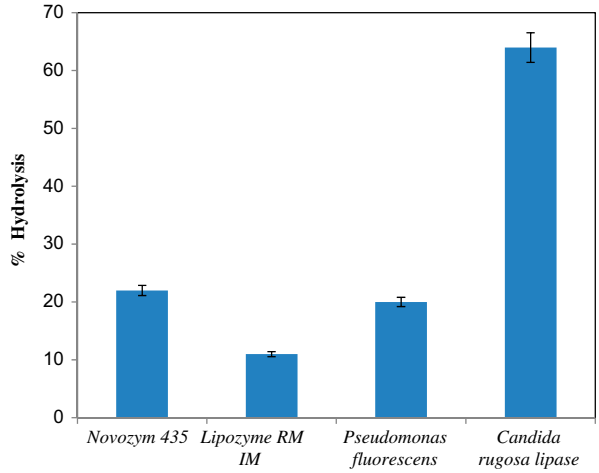

Fig. 1. Screening of different lipases at enzyme loading 0.2% w/v, NaCl concentration 100 mM, temperature 30°C, fat concentration 2,000 mg/mL and agitation speed 200 rpm.

3.2. Effect of reaction time

The effect of reaction time on hydrolysis of the synthetic dairy wastewater using lipase *Z* was investigated at around 30 ± 2°C. It can be seen from Fig. 2 that although hydrolytic reaction at first increased linearly with time, but the rate reduced considerably as the reaction progressed. The concentration of the free fatty acids formed as the result of the reaction increased swiftly at the initial 14 h to 70.5%, and then very gradually to 74.7% at 24 h and after that it remained almost constant. It could be concluded that at first, the interaction between enzyme and substrate was ample due to availability of more enzyme. However, with the further progression, there is possibility that product formed are blocking the active sites of the enzyme [14,15]. It is also predicted that the enzyme seems to be not very active at acidic pH and all of these resulted in a decreased rate of reaction with time.

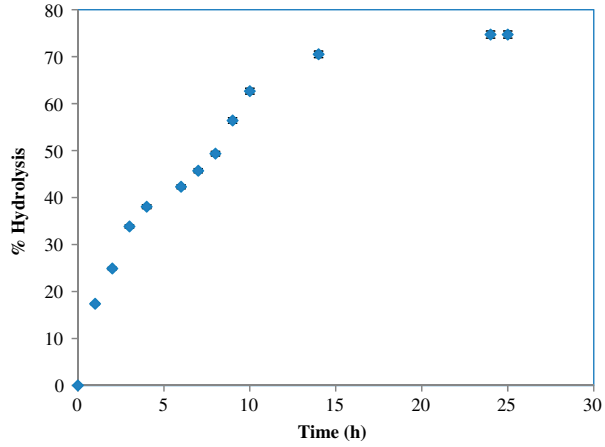

Fig. 2. Effect of reaction time on hydrolysis at enzyme loading 0.2% w/v, NaCl concentration 100 mM, temperature 30˚C, fat concentration 2,000 mg/mL and agitation speed 200 rpm.

3.3. Effect of enzyme loading

The enzyme is an important factor economically for its commercial application. This effect was studied by varying enzyme loading as 0.1, 0.2, 0.4, 0.6 and 0.8% (w/v) with respect to dairy wastewater used. The temperature of the reaction was kept at around 30˚C and 100 mM of NaCl was added as an emulsifying aid. Initially, the conversion of fats to free fatty acid increased from 0.1 to 0.4% w/v enzyme loading i.e. 63.44–77.5% (Fig. 3). On the other hand, further increased in loading from 0.6 to 0.8% w/v resulted in decreased conversion i.e. 70.4–64.85%. Though the initial reaction rate was higher at 0.6% w/v of the enzyme than at 0.2 and 0.4% w/v, the final conversion was found to be lower than these two concentrations. The quick enzyme–substrate complexes formation at higher enzyme loading may led to higher initial reaction and more product formation. However, the formation of higher product at start may result in blockage of the active sites at faster rate owing to the product accumulation leading to decrease in rate as well as conversion. Nevertheless, excess enzyme caused difficulties in assimilation by increased viscosity and thus, hindering the mass transfer [16].

3.4. Effect of temperature

Sensitivity and reactivity of enzymes are mostly dependent on reaction temperature. Thus, the effect of temperature on the hydrolysis reaction was studied in the range from 20 to 60˚C with the optimized 0.2% w/v enzyme loading as discussed above and 100 mM of NaCl. The results are depicted in Fig. 4. Generally, the dissolution rate of substrates and intermediate is restricted by lower reaction temperatures; at the same time moderate to high reaction temperatures augment the reaction rate by decrease in the viscosities of the reaction medium and shortening the reaction time. Here, reaction temperature of 20–30˚C, showed increase in the initial reaction rate along with the conversion from 60.6 to 74.7%. However, the conversion obtained after 24 h for a reaction temperature range of 40–60˚C decreased from 73.3 to 52.75% (Fig. 4). This can be due to the thermal denaturation of the lipase taking place at

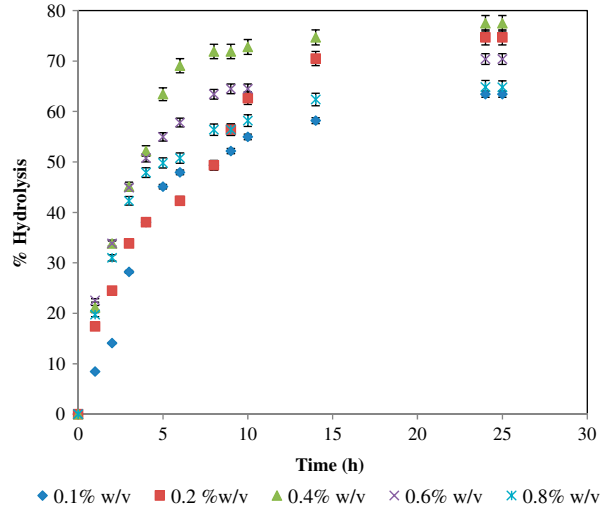

Fig. 3. Effect of enzyme loading on fat hydrolysis at NaCl concentration 100 mM, temperature 30˚C, fat concentration 2,000 mg/mL and agitation speed 200 rpm.

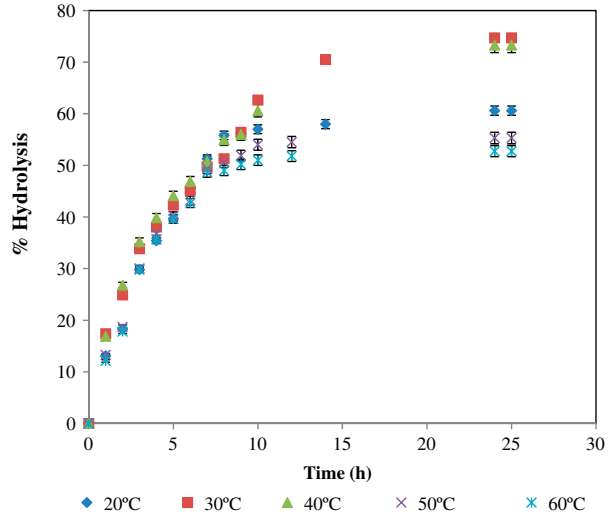

Fig. 4. Effect of temperature on fat hydrolysis at enzyme loading 0.2% w/v, NaCl concentration 100 mM, fat concentration 2,000 mg/mL and agitation speed 200 rpm.

higher temperature. At higher temperature, the bonds maintaining the active enzyme conformation are disrupted and thereby, decreasing the conversion [16,17]. Therefore, the optimum temperature selected was 30°C, since it was confirmed that at this temperature the enzyme activity was higher and maximum conversion was obtained.

3.5. Effect of fat concentration

The normal fat content in the milk processing unit was reported to be in the range of 800–1,200 mg/mL and depending upon the product manufactured or process used, the fat content in the dairy wastewater would vary accordingly. Hence, effect of initial fat concentration was studied to determine the activity of enzyme on different fat content. Fat content in the range of 2,000–8,000 mg/mL (i.e. 2,000, 4,000, 6,000 and 8,000 mg/mL) were evaluated. The enzyme concentration and temperature were kept at optimized conditions i.e. 0.2% w/v and 30°C. It can be seen from Fig. 5 that as the concentration of the fats increases, the conversion is eventually decreased.

This may be due to the reason that the molecules of the enzyme (lipase Z) are coated by the fat molecules as the fat concentration increases. Thus, rendering them ineffective towards the hydrolytic reaction and thus decrease in the rate of reaction as well as the overall conversion. Similarly, the amount of fatty acids formed is constant as the same enzyme loading is used for all the fat concentration which resulted in decrease in % hydrolysis with increase in fat acid concentration. It was found that as the fat content increases from 2,000 to 8,000 mg/mL, the conversion decreased from 74.7 to 56.75%. Though it can be said that the decrease is not very much substantial looking at the very high concentration of the fat and moreover it is noticed that normally the milk processing unit has fat content of less than 2,000 mg/mL. It can also be observed that the enzyme do act effectively with increased concentration of the FOGs.

3.6. Effect of NaCl concentration

Though lipase showed potential applications in degrading oil and fats in wastewater generated by dairy industries, emulsifying agents or aids have been steadily used to quantify lipase activity, majorly to amplify the lipid–water interfacial area, as a result of which the observed rate of lipase catalysed reaction is enhanced [12]. NaCl neutralizes the negative pH gradient at the interface (created due to attraction of negative charged hydrogen ions by the fatty acids formed) by reducing the critical micelle concentration value and increasing micelles size and thus enhancing the lipase activity [12]. It was seen that regardless the lipase, runs using NaCl gave higher fatty acid concentrations than runs without the presence of NaCl (Fig. 6). Further, different concentrations of NaCl ranging from 100 to 800 mM were evaluated to determine the concentration at which it yielded maximum conversion. The reactions were carried out using

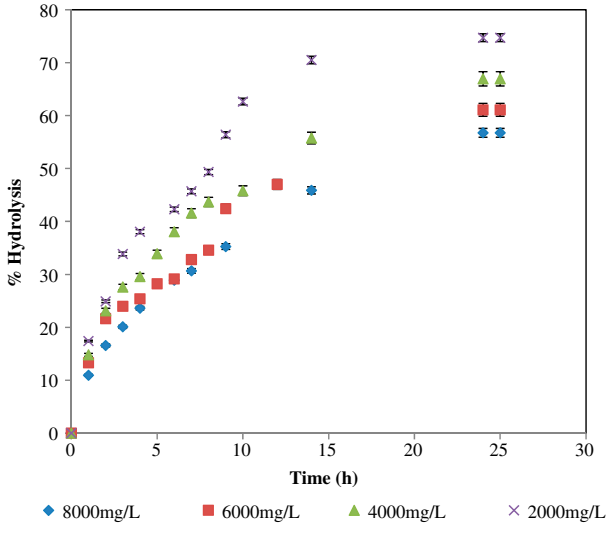

Fig. 5. Effect of different fats concentration on fat hydrolysis at enzyme loading 0.2% w/v, NaCl concentration 100 mM, temperature 30°C and agitation speed 200 rpm.

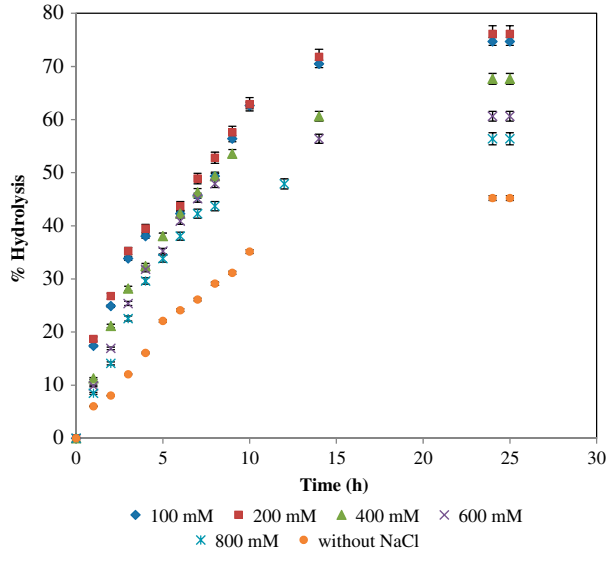

Fig. 6. Effect of different NaCl concentrations on fat hydrolysis at enzyme loading 0.2% w/v, temperature 30°C, fat concentration 2,000 mg/mL and agitation speed 200 rpm.

0.2% w/v of enzyme and 30˚C. It was seen that the hydrolysis increased up to 200 mM i.e. from 74.7 to 76.13% then, it decreased gradually by further NaCl concentration from 67.67 to 56.39% (Fig. 6).

Though the initial reaction rate and conversion is higher at 200 mM than 100 mM but the difference is not very significant; however, the concentration is doubled and hence 100 mM was selected as the optimized concentration of NaCl.

3.7. Characterization of treated dairy wastewater

The COD of the crude dairy wastewater (before pre-treatment) was found to be around 42,000 ppm which was reduced to 12,000 ppm when the wastewater was treated at the optimized reaction conditions and parameters as discussed above. Thus, it could be said that after the enzymatic pre-treatment of the synthetic dairy wastewater using lipase *Z*, 72% of COD removal was observed.

4. Conclusion

The present work evaluates the enzymatic pre-hydrolysis of synthetic dairy wastewater using lipase *Z* prior to the anaerobic degradation. The fat content of the dairy wastewater was kept at around 2,000 mg/mL. The various process parameters affecting the rate of this enzyme catalysed pre-hydrolysis reaction were investigated. As justified by the results, the enzyme loading was selected to be 0.2% w/v and a temperature of 30˚C was selected to be optimal as above this temperature the yield decreased subsequently due to instability of the enzyme at higher temperature owing to its thermal denaturation. NaCl which was used as an emulsifying aid improved the enzyme activity and thus the rate of reaction by around 30% as it provides appropriate reaction condition required for the interface activation where the actual lipase reaction takes place. The total amount of NaCl required was also optimized. Under optimal reaction conditions, reaction conversion of 75% was observed along with 72% COD removal.

References

[1] R.U. Rani, J.R. Banu, S.A. Kumar, S. Kaliappan, I.T. Yeom, Low temperature thermo-chemical pretreatment of dairy waste activated sludge for anaerobic digestion process, Bioresour. Technol. 103 (2012) 415–424.

[2] J. Kushwaha, V. Srivastav, I. Mall, An overview of various technologies for the treatment of dairy wastewaters, Crit. Rev. Food Sci. Nutr. 51 (2011) 442–452.

[3] B. Demiral, O. Yenigun, T. Onay, Anaerobic treatment of dairy wastewaters: A review, Process Biochem. 40 (2005) 2583–2595.

[4] W. Janczukowicz, M. Zieliński, M. Debowski, Biodegradability evaluation of dairy effluents originated in selected sections of dairy production, Bioresour. Technol. 99 (2008) 4199–4205.

[5] E. Qamsari, R. Karmanshahi, M. Nosrati, T. Amani, Enzymatic pre-hydrolysis of high fat content dairy wastewater as a pretreatment for anaerobic digestion, Int. J. Environ. Res. 6(2) (2012) 475–480.

[6] F. Jung, M.C. Cammarota, D.M.G. Freire, Impact of enzymatic pre-hydrolysis on batch activated sludge systems dealing with oily wastewaters, Biotechnol. Lett. 24 (2002) 1797–1802.

[7] M. Leal, D. Freire, M. Cammarota, G. Sant Anna Jr., Effect of enzymatic hydrolysis on anaerobic treatment of dairy wastewater, Process Biochem. 41 (2006) 1173–1178.

[8] M.P. Prasad, K. Manjunath, Comparative study on biodegradation of lipid rich wastewater using lipase producing bacterial species, Indian J. Biotechnol. 10 (2011) 121–124.

[9] M.K. Stenstrom, S. Fam, G.S. Silverman, Analytical methods for quantitative and qualitative determination of hydrocarbons and oil and grease in water and wastewater, Environ. Technol. Lett. 7 (1986) 625–636.

[10] E. Pereira, A. Mendes, A. Furigo Jr., H. Castro, Anaerobic biodegradability of dairy wastewater pretreated with porcine pancreas lipase, Braz. Arch. Biol. Technol. 53 (2010) 1279–1284.

[11] W. Adam, M. Lazarus, C. Saha-Mollera, O. Weichold, U. Hoch, D. Haring, P. Schreie, Biotransformation with peroxidase, Adv. Biochem. Eng. Biotechnol. 63 (1999) 73–107.

[12] A. Mendes, H. Costra, Effect on the enzymatic hydrolysis of lipids from dairy wastewater by replacing gum Arabic emulsifier for sodium chloride, Braz. Arch. Biol. Technol. 48 (2005) 135–142.

[13] L. Masse, K. Kennedy, S. Chou, Testing of alkaline and enzymatic pre-treatment for fat particles in slaughterhouses wastewater, Bioresour. Technol. 77 (2001) 145–155.

[14] M.S. Puthli, V.K. Rathod, A.B. Pandit, Enzymatic hydrolysis of castor oil: Process intensification studies, Biochem. Eng. J. 31(1) (2006) 31–41.

[15] V.K. Rathod, A.B. Pandit, Effect of various additives on enzymatic hydrolysis of castor oil, Biochem. Eng. J. 47(1–3) (2009) 93–99.

[16] N. Gharat, V.K. Rathod, Enzyme catalyzed transesterification of waste cooking oil with dimethyl carbonate, J. Mol. Catal. B: Enzym. 88 (2013) 36–40.

[17] S.M. Radzi, W.A.F. Mustafa, S.S. Othman, H.M. Noor, Green synthesis of butyl acetate a pineapple flavour in a lipase catalyzed reaction, W.A.S.E.T. 59 (2011) 677–680.