Indian Journal of Biotechnology
Vol. 17, April 2018, pp 244-250

Effect of lipase from different source on high fat content wastewater of dairy industry

Vivek P Bhange and Sanvidhan G Suke*
Department of Biotechnology, Priyadarshini Institute of Engineering and Technology, Nagpur 440019, India

Received 5 March 2014; revised 28 July 2016; accepted 4 August 2016

Wastewaters of dairy industry usually present high fat contents. The present study was designed to investigate the effect of different sources of lipase such as lipase contains raw milk, crude lipase from groundnuts extract, fat degrading microorganism from up flow anaerobic sludge blanket reactor (UASBR) culture in nutrient broth and lipase producing microorganism from UASBR culture in a selective media inoculated in holding tank dairy wastewater. Pretreatment of 250 ml dairy wastewater with 10 ml lipase contain sample was optimized under anaerobic condition for 72 hrs at 37ºC. Analytical parameters pH, fat content (FC), chemical oxygen demand (COD) and total solids (TS) were analysed in each treatment process. The results showed that pH of the samples were maintained, the UASBR culture inoculated in selective media degraded the maximum amount of fat; similarly maximum amount of COD and TS were reduced in the selective media treated sample as compared to others treatment process. This study illustrated that application of an enzymatic pretreatment process to hydrolyze and dissolve fats may improve the biological degradation of high fat content in wastewaters. Moreover, pretreatment of wastewater from different lipase sources are new and promising application for lipases. Thus, it is apparent that use of this enzymatic biological treatment can serve an alternative for treatment of Soybean casein digest Soybean casein digest Soybean casein digest Soybean casein digest fat containing wastewater and to provide pollution free environment.

Keywords: Dairy wastewater, lipase, fat content, anaerobic condition, microorganisms

Introduction

A continually rising the demand for milk and its products in many countries has led to advancements, which has subsequently led to steady growth in the production of milk per head of cattle. This has caused enormous growth of dairy industries in most of the states of the India. Consequently, the amount of wastewater generated and discharged from these industries has also increased. Industrial wastewater may adversely affect the water bodies when not appropriately treated[1-5]. Wastewaters from dairies are rich in biodegradable organic molecules and nutrients that usually contain high levels of fats and proteins which have a low biodegradability coefficient[6-8]. The high fat content milk and its products can also cause physical problems within drainage systems. Solidified fat can cause blockages, resulting in overflows from the system and possible pollution of watercourses. If not treated, they cause gross pollution of land and water with their high biochemical oxygen demand (BOD) and chemical oxygen demand (COD). Hence, strict guidelines have been established by government agencies to prevent water contamination[9-10]. It is necessary to monitor the wastewater properly before discharge.

Fats consist of a wide group of compounds that are generally soluble in organic solvents and largely insoluble in water. Chemically, fats are generally tri-esters of glycerol and fatty acids. Fats may be either solid or liquid at room temperature, depending on their structure and composition. Lipids used to refer to both liquid and solid fats, along with other related substances[11]. Lipids are an important component in wastewater that causes severe environment pollution. It can form oil films on water surfaces, preventing the diffusion of oxygen from air into water, leading to the death of many forms of aquatic life. Aggregates formed by oil droplets and other particles present in wastewater can also block water drainage lines. The application of anaerobic reactors to treat lipids-rich wastewater can cause granular sludge flotation and wash-out, inhibition of methanogenic activity and decrease the concentration of adenosine triphosphate[12-13]. To cope with these negative effects on the water stream treatment, several

*Author for correspondence:
Tel: +91-7104-236463; Fax: +91-7104-236458
sgsuke@hotmail.com

strategies for removing lipids residues at the head of the plant are proposed. Among these, the alternative of using specific enzymes (lipases) has, recently, potentially gained more attention because of stringent environmental regulations and clean and friendly application of enzymes[14-15].

Lipases (triacylglycerol acyl hydrolases, E.C. 3.1.1.3) are enzymes that catalyze the hydrolysis of triacylglycerols to glycerol and free fatty acids at the water-lipid interface and the reverse reaction in non-aqueous media[16]. These enzymes showed potential applications in degrading oil and fats in wastewater generated by dairy industries, slaughterhouses, edible oils, fat refineries and others[17]. The particular benefits offered by enzymes are specificity, mild conditions and reduced waste. It may be possible, by choosing the right enzyme, to control which products produced, and unwanted side reactions are minimized due to specificity of enzymes that appear in the waste stream. The plant using enzymatic reactions can be built and operated at much lower capital and energy cost. Enzyme-based processes tend to have lower waste treatment costs. However, enzymes are biodegradable, and since they usually are closed at 0.1 - 1.0% of substrate, the contribution of the enzyme to the BOD in the waste stream is negligible[18].

To alleviate the problem, aerobic and anaerobic treatments mainly are used, but in the last two decades anaerobic reactors have been increasingly used[19]. Application of anaerobic treatment is widespread in food and agro industries. However, there are some concerns about its capability to assimilate variable loads of oil and grease. The operational problems caused by oil and grease in up-flow anaerobic sludge blanket (UASB) reactors, such as sludge flotation, inhibitory and toxic effects of intermediate products were reported[20]. Detrimental effects of milk fat on anaerobic treatment were also reported[21]. These reports considered that the loss of process performance can be attributed to the low rate of fat hydrolysis in the anaerobic reactor. A large number of pretreatment systems are employed to remove oil and grease from these wastewaters prior to the main treatment process itself, which is generally of a biological nature. However, the cost of such reagents is high, the removal efficiency of dissolved and/or emulsified oil and grease is low and extremely problematic sludge can be produced[22-23].

The present study is therefore, aimed at evaluating the effect of lipase from different sources in anaerobic condition on fatty wastewater of dairy industry of central India.

Materials and methods

Materials

Dairy wastewater samples were collected from dairy industry located in the Maharashtra state of central India and stored in refrigerator at a temperature 4ºC in laboratory for further use. Dairy industry effluent treatment plant lay out shown in Figure 1. Groundnuts for lipase extraction were purchased from local market of Nagpur city Nagpur, India. All chemicals used to prepare the growth medium for lipase were procured from Hi-Media, Mumbai, India. Similarly chemicals for analysis were purchased from Loba Chemie Pvt. Ltd., and S. D. Fine Chem Ltd, Mumbai, India.

Analytical Methods

Dairy wastewater samples and different sources of lipase with their activity were measured by titrimetric method[24]. Wastewater treatment were analysed for fat content (FC) by the method of Rose-Gottlieb method[25], COD evaluated by the method of Eaton and Franson[26] and total solid (TS) was determined according to the standard methods[27]. The pH was measured using the Systronic 362 pH meter.

Experimental Procedures

Treatment With Raw Milk as Lipase Source

Unscreened holding tank sample collected in three 250 ml conical flask. First flask contained 250 ml of holding tank dairy milk wastewater as blank sample (without raw milk), in the second flask 250 ml of raw milk, whose fat was removed manually and added. In the third flask 250 ml of wastewater and 10 ml raw milk sample was added[28]. All three flasks were kept at temp 37 - 40ºC for five days in anaerobic conditions by covering its top by cotton and aluminium foil. All above analytical parameters (pH, FC, COD and TS) were estimated daily for five days. One gram of caustic of what was added on the second day and 0.5 gm on the fourth day to both the samples contained raw milk to maintain the pH.

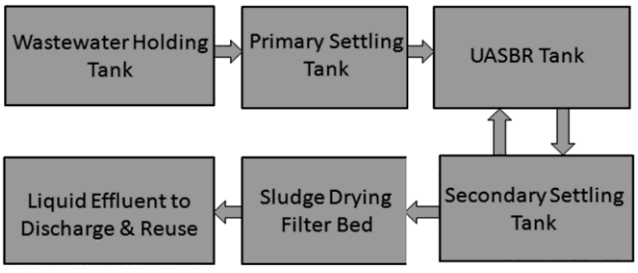

Fig. 1 — Dairy wastewater effluent treatment plant layout.

Treatment With Enzyme Lipase Crude Extract from Groundnuts

First lipase was extracted from groundnuts in crude enzyme form by applying method described by Huang *et al*[29]. The pre-estimated holding tank sample was placed in a 250 ml conical flask and inoculated with 10 ml of crude lipase extract. To provide anaerobic conditions, the conical flask's top was covered with cotton plug and the entire surface of the flask wrapped with aluminium foil to prevent diffusion of gases into the flask from outer surroundings. The sample then incubated at 37 - 40ºC, for 72 hrs and at end of third day all above mentioned analytical parameters were measured.

Inoculation with Fat Degrading Microorganism from UASBR Culture in Nutrient Broth

Fat rich wastewater sample were collected from holding tank in the sterile container. For isolation of fat degrading microorganism, 10 ml UASBR culture was collected from dairy plant in 100 ml double distilled water. The serially diluted (10^{-1} to 10^{-6}) samples were placed on nutrient agar plates. The formation of clear zone around the colony on the plate considered as fat degrading lipolytic microbes[30]. Hence, we decided to confirm the presence of fat degrading microorganism in the UASBR culture. Moreover, nutrient broth containing peptone, sodium chloride, buffer solution and yeast extract were placed in appropriate proportion and known quantity of distilled water added to it and autoclaved[31]. After autoclaving the broth, cream was added as an initiation factor for the microorganisms to grow. Removed the UASBR culture sample and inoculated 10 ml of culture into 250 ml of flask contained holding tank sample. The inoculated sample was incubating for 48 to 72 hrs at 37ºC in anaerobic condition, by closing the top of the flask with cotton plug and then wrapping the complete flask by aluminium foil. Before (day 1) and on the third day of incubation of the UASBR culture with the unscreened holding tank wastewater sample were collected and all the parameters (pH, FC, COD and TS) as mentioned above were estimated.

Inoculation with Lipase Producing Microorganism from UASBR Culture in a Selective Media

The lipase producing selective medium contained 1.3 g/l ammonium sulphate [$(NH_4)_2SO_4$], 0.9 g/l dipotassium hydrogen phosphate (K_2HPO_4), 0.6 g/l potassium dihydrogen orthophosphate (KH_2PO_4), 0.2 g/l magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), 0.1 g/l yeast extract, 0.5 g/l calcium carbonate ($CaCO_3$) and 10 gm/l of cream[32]. These components were weighed in proper proportion in order to prepare 250 ml of selective media and the volume was made up by adding distilled water, 10 ml UASBR pre cultured sample were collected in a beaker. It was then inoculated in the selective media and incubated in the incubator for 48 to 72 hrs at 37 to 40ºC in anaerobic condition. Collected 10 ml selective media UASBR inoculated with 250 ml holding tank sample flask and incubated in anaerobic condition for 72 hrs at 37ºC. All above mentioned analytical parameters (pH, FC, COD and TS) were measured before (day 1) and on the 3rd day of incubation of inoculated sample.

Analytical Experiment of Efficiency Study Wastewater Treated Sample to Degrade Fat

After completion of all the pre-activities of inoculation, five 250 ml of conical flasks containing 250 ml of holding tank dairy wastewater sample were taken. First flask consider as bank sample, in the second conical flask 10 ml of raw milk sample was added; in the third flask 10 ml of crude lipase extract was added, similarly 10 ml of UASBR culture in normal nutrient broth and 10 ml UASBR culture in selective media were added in the respective flasks. These entire experimental flasks were made anaerobic by covered the top with cotton plugs and wrapped them completely by aluminium foil, and incubated for 72 hrs at 37- 40ºC temperature. Before and after 72 hrs, the entire setup was removed from the incubator, and all the parameters were estimated again as initial and final reading.

Comparative Study for Different Concentrations of UASBR Culture Inoculated in Wastewater

The UASBR culture was inoculated in the selective media in the order of 5%, 10% and 15% culture concentration i.e. 50 ml selective culture was added in 1000 ml of holding tank sample for 5% concentration; similarly, 100 ml and 150 ml selective media culture were added in 1000 ml of holding tank sample for 10% and 15% concentration, respectively and incubated all three samples at 37- 40ºC for 72 hrs under anaerobic condition (as mentioned earlier). Before and after 72 hrs in these three concentration of sample, all above analytical parameters were estimated as initial (1st day) and final (3rd day) readings.

Data Analysis

All mathematical and statistical calculations were implemented using Microsoft Office Excel 2007.

Results and Discussion

Pretreatment of dairy wastewater to remove fats and reduced the COD and TS using physical and/or chemical methods are largely limited due to poor efficiency of the methods, high cost, or both. Although biological methods using microorganisms and/or their products such as hydrolytic enzymes are usually preferred, the major problem with such methods is that the hydrolysis products of fats present in wastewater are mainly long chain fatty acids which are potentially toxic to several microorganisms commonly employed in secondary treatment of such high strength wastewaters[33-34]. Present study designed to investigate the effect of enzyme lipase obtains from different source treated with high fat content dairy wastewater. We found that the maximum lipase activity occurred in the microbial medium as compared to raw milk and vegetable oil (data not shown).

Previous report of the research on various uses of lipase enzyme stated that the lipases are ubiquitous enzymes with considerable industrial potential since they have not only general advantages of biocatalysts, such as high catalytic activity, mild reaction conditions, environmental friendliness, and exquisite chemical, enantio and regio selectivity, but also very broad substrate range and excellent stability[35]. Previous study shows that raw milk contains the lipolytic enzymes which are responsible for hydrolysis of natural glycerol fat[36]. In the present study holding tank dairy wastewater treated with raw milk (Table 1), initial fat content (FC) of treated sample 14350 ppm and COD 3633 mg/l, TS 5220 mg/l on day first. After completion of incubation period at 5th day FC, COD and TS are reduced 1560 ppm, 1960 mg/l and 2430 mg/l, respectively. Appropriate amount of caustic was added in the sample to maintain the pH.

Now a day's many plant lipases have been isolated which can be used for the production of important lipases enzymes[37]. Moreover, lipases are present in the vegetable oil[38]. Present study Table 2 shows that extracted groundnuts crude lipase enzyme treated with dairy wastewater for three days, on first day pH was 12 and after third day pH 9, fat content was decreased remarkably from 7600 ppm to 1200 ppm, the COD along with the TS content were also reduced from 4528 to 3521 mg/l and 5500 to 2200 mg/l, respectively.

Microbial enzymes are often more useful than enzymes derived from plants or animals because of the great variety of catalytic activities available, the high yields possible, ease of genetic manipulation, regular supply due to absence of seasonal fluctuations and rapid growth of microorganisms on inexpensive media. Microbial enzymes are also more stable than their corresponding plant and animal enzymes and their production is more convenient and safer[37]. UASB reactors have been widely used for the anaerobic dairy wastewater treatment in full-scale applications[9,39]. The basic elements of a typical UASB reactor are a sludge blanket, influent distribution system, gas-solid separator, and the effluent withdrawal system. Previous report shows that the treatment of dairy wastewaters containing elevated fat in a UASB reactor resulted in effluents of high turbidity, volatile suspended solids and COD removal 50% less amount[6]. In the present study, up flow anaerobic sludge blanket reactor (UASBR) was considered and to confirm the presence of fat degrading microorganism in the UASBR culture.

Table 2 — Effect of pH, FC, COD and TF on hydrolysis using extracted crude enzyme lipase from groundnuts inoculated in holding tank wastewater sample

Days	pH	FC (ppm)	COD (mg/l)	TS (mg/l)
Day 1	12	7600	4528	5500
Day 3	9	1200	3521	2200

Table 1— Effect of pH, FC, COD and TS on dairy wastewater treated with lipase enzyme source from raw milk

Days	Sample	pH	FC (ppm)	COD (mg/l)	TS (mg/l)
Day 1	Bank	11.4	7600	4550	6400
	Raw milk	6.4	6750	3847	4260
	Wastewater + Raw milk	8.3	14350	3633	5220
Day 2	Wastewater + Raw milk	5.3	7400	3620	4600
		(Addition of 1 gm caustic)			
Day 3	Wastewater + Raw milk	7.2	4360	3160	2800
Day 4	Wastewater + Raw milk	6.9	3650	2930	2760
		(Addition of 0.5 gm caustic)			
Day 5	Wastewater + Raw milk	8.9	1560	1960	2430

Nutrients agar was taken for plating the culture and incubates for 72 hrs at 37ºC. After obtaining the colonies on the nutrient agar it was subjected to the Gram staining and the result was obtained cocci shaped Gram positive colonies (result not shown). Microbes which formed large clear zone around the colony based on morphological, biochemical and physiological characters according to Bergey's Manual of Determinative Bacteriology[40]. As an alternative approach to pretreated dairy wastewater, in the present study isolated fat degrading microorganism from UASBR culture in nutrient broth and inoculated in holding tank wastewater sample. From the Table 3 it was observed that after 72 hrs, the fat content was decreased remarkably from 6320 ppm to 1160 ppm, the COD and the TS content were also reduced at day 3 as compared to first day. Previous report of all bacterial lipase states that maximum activity of lipases at pH values 7 has been observed in many cases[41]. Pure cultures of lipases producing organisms were maintain on nutrient agar slant and prepared UASBR subculture in the selective media and inoculated in holding tank wastewater. Table 4 shows that the degradation of fat up to 50% was obtained within a period of 72 hrs. There was reduction in COD and TS to satisfy the statutory requirements of dairy effluent requirements stated by Maharashtra Pollution Control Board and Central Pollution Control Board (CPCB) of India[9,42].

Based on above, for efficacy study again we performed all samples together by inoculating dairy wastewater sample with lipase obtained from different sources. Fig. 2 indicated that pH of the sample was maintained; the UASBR culture inoculated in selective media degraded the maximum amount of fat in the minimum period of time as compared to others. Similarly COD and TS were reduced maximal in the selective media treated sample as compared to others.

Table 3 — Effect of pH, FC, COD and TS on hydrolysis using isolated fat degrading microorganism from UASBR culture in nutrient broth inoculated in holding tank wastewater sample

Days	pH	FC (ppm)	COD (mg/l)	TS (mg/l)
Day 1	11	6320	4420	5220
Day 3	9	1160	2320	1940

Table 4 — Effect of pH, FC, COD and TS on hydrolysis using isolated lipase producing microorganism from UASBR culture in selective media inoculated in holding tank wastewater sample

Days	pH	FC (ppm)	COD (mg/l)	TS (mg/l)
Day 1	11	1000	4630	4530
Day 3	8	500	240	1196

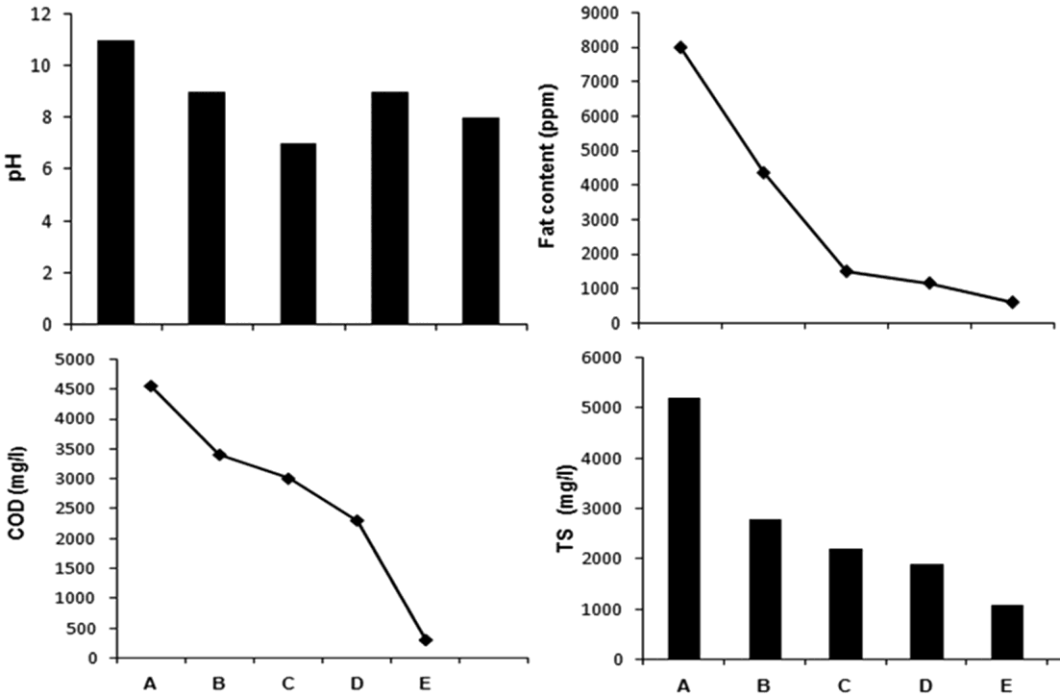

Fig. 2 — Effect of different source of lipase on pH, FC, COD and TS in inoculated dairy wastewater sample. A: Holding tank dairy wastewater blank sample, B: Raw milk treated sample, C: Crude lipase extract treated sample, D: UASBR culture in nutrient broth treated sample and E: UASBR culture in selective media sample.

Table 5 — Comparative study for different concentrations of UASBR culture in selective media inoculated with dairy wastewater of holding tank

Concentration	Reading	pH	FC (ppm)	COD (mg/l)	TS (mg/l)
5 %	Initial	11.2	6730	5760	2200
	Final	6.59	200	2000	1500
10 %	Initial	11.26	7500	4550	2560
	Final	6.69	200	1600	1920
15 %	Initial	11.26	6920	4760	2240
	Final	7.78	200	1400	900

Considering the above analytical study, UASBR culture inoculated in selective media degraded the maximum amount of fat as compared to others. However, for the same reason we decided to further study the concentration variance of selective media sample when inoculated in the unscreened wastewater from the holding tank sample. Table 5 indicated that inoculation of wastewater with three different (5%, 10% and 15%) concentrations of UASBR culture in selective media. The results shows that concentration variance, 15% of UASBR culture inoculated in selective media proved to be best suited for the degradation of fat from the dairy wastewater. As the fat degradation in 15% concentration sample was more than 50% and the other parameters such as pH, COD and TS content were under the desirable limits.

The results of degradation of fat from the above experiments indicated that very different degradation efficiency might be due to the different reaction system of lipase from each source. Lipase present not only catalyzed hydrolysis reaction but also catalyzed inter esterification reaction, depending on the source of lipase and reaction condition. Therefore, in the mixed culture system of the present study hydrolysis reaction occurred but inter esterification reaction had not determined either some reaction or occurred on both reactions. Hence, isolated lipase producing bacteria were suggested to be used in wastewater treatment which will increased wastewater treatment efficiency.

Conclusion

The present study suggest that the lipase enzyme present in the raw milk and crude enzyme extract efficiently degrades the fat and reduced the COD, TS and also balances the pH from the dairy wastewater. UASBR culture contained microorganism that degraded the fat, under anaerobic condition in minimum duration of time. UASBR culture inoculated in selective media was considered best for further study of degradation of fat in dairy industry wastewater. Thus, it is apparent that the use of this enzymatic biological treatment can serve an alternative for treatment of high fat containing wastewater and to provide clean pollution free environment.

Acknowledgement

We thankful to the support by Lokmanya Tilak Janakalyan Shikshan Santha's (LTJSS), Nagpur for completion of this work.

References

1 Valh V, Majcen Le J, Marechal A, Križanec B & Vajnhandl S, The Applicability of an advanced oxidation process for textile finishing waste streams and fate of persistent organic pollutants, *Int J Environ Res*, 6 (2012) 863–874.

2 Rameshraja D & Suresh S, Treatment of tannery wastewater by various oxidation and combined processes, *Int J Environ Res*, 5 (2011) 349–360.

3 Houda B, Dorra G, Chafai A, Emna A & Khaled M, Impact of a mixed industrial and domestic wastewater effluent on the southern coastal sediments of Sfax (Tunisia) in the mediterranean sea, *Int J Environ Res*, 5 (2011) 691–704.

4 Naim R, Kisay L, Park J, Lee C, Qaisar M, Zulfiqar A B & Noshin M, Precipitation chelation of cyanide complexes in electroplating industry wastewater, *Int J Environ Res*, 5 (2012) 85–92.

5 Daniel R, & Prabhakara Rao A V S, An efficient removal of arsenic from industrial effluents using electro-coagulation as clean technology option, *Int J Environ Res*, 6 (2012) 711–718.

6 Cammarota M C, Teixeira G A & Freire D M G, Enzymatic prehydrolysis and anaerobic degradation of wastewaters with high fat contents, *Biotechnol Lett*, 23 (2001) 1591–1595.

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

8 Omil F, Garrido J M, Arrojo B & Méndez R, Anaerobic filter reactor performance for the treatment of complex dairy wastewater at industrial scale, *Wat Res*, 37 (2003) 4099–4108.

9 Kushwaha J P, Srivastava V C & Mall I D, An overview of various technologies for the treatment of dairy wastewaters, *Criti Rev Food Sci Nutri*, 51 (2011) 442–452.

10 Pattnaik R, Yost R S, Porter G, Masunaga T & Attanandana T, Improving multi-soil-layer (MSL) system remediation of dairy effluent, *Ecol Eng*, 32 (2008) 1–10.

11 Mendes A A & Castro H F, Effect on the enzymatic hydrolysis of lipids from dairy wastewater by replacing gum arabic emulsifier for sodium chloride, *Braz Arch Biolo Technol*, 48 (2005) 135–142.

12 Perle M, Kimchie S & Shelef G, Some biochemical aspects of the anaerobic degradation of dairy wastewater, *Wat Res*, 29 (1995) 1549–1554.

13 Mendes A A, Avaliação da biodegradabilidade anaeróbia de efluentes com alto teor de lipídeos previamente tratados com enzimas hidrolíticas. MSc Dissertation, Chemical Engineering College of Lorena, Lorena, Brazil, 2004.

14 Gandhi N N, Applications of lipases, *J Am Oil Chem Soc*, 74 (1997) 621–634.

15 Mendes A A, Castro H F, Pereira E B & Furigo Jr A, Aplicação de lipases no tratamento de águas residuárias com elevado teor de lipídeos, *Quim Nova*, 28 (2005) 296–305.

16 Castro H F, Mendes A A, Santos J C & Aguiar C L, Modificação de óleos e gorduras por biotransformação, *Quim Nova*, 27 (2004) 146–156.

17 Masse L, Kennedy K J & Chou S, Testing of alkaline and enzymatic pretreatment for fat particles in slaughterhouses wastewater, *Bioresour Technol*, 77 (2001) 145–155.

18 Posorske L H, Industrial scale application of enzymes to the fat and oil's industry, *J Am Oil Chem Soc*, 61 (1984) 1758–1760.

19 Omil F, Garrido J M, Arrojo B & Méndez R, Anaerobic filter reactor performance for the treatment of complex dairy waste-water at industrial scale, *Wat Res*, 37 (2003) 4099–4108.

20 Vidal G, Carvalho A, Me´ndez R & Lema J M, Influence of the content in fats and proteins on the anaerobic biodegradability of dairy wastewaters, *Bioresour Technol*, 74 (2000) 231–239.

21 Petruy R & Lettinga G, Digestion of a milk-fat emulsion, *Bioresour Technol*, 61 (1997) 141–149.

22 Tano-Debrah K, Fukuyama S, Otonari N, Taniguchi F & Ogura M, An inoculum for the aerobic treatment of wastewaters with high concentrations of fats and oils, *Bioresour Technol*, 69 (1999) 133–139.

23 Willey R, Fats, oils and greases: the minimization and treatment of wastewaters generated from oil refining and margarine production, *Ecotox Environ Safe*, 50 (2001) 127–133.

24 Jensen R G, Detection and determination of lipase (acylglycerol hydrolase) activity from various sources, *Lipids*, 18 (1983) 650-657.

25 Association of Official Analytical Chemists, (AOAC), Official Method of Analysis, 15th Ed., The Association of Official Agricultural Chemists, Virginia, 1990, 10-18.

26 Eaton A D & Franson M A H, Standard methods for the examination of water and wastewater, American Public Health Association, 2005, 1200.

27 APHA, AWWA & WEF, Standard methods for the examination of water and wastewater, 19th Ed., (Publication Office APHA: Washington DC) 1998.

28 Delgado F J, Gonzalez-Crespo J, Ladero L, Cava R & Ramirez R, Free fatty acid and oxidative changes of a panish soft cheese (PDO Tortadel Casaŕ) during riping, *Int J Food Techno*, 44 (2009) 1721–1728.

29 Huang A H C, Wimer I T & Lin Y, Lipase in the lipid bodies of corn scutella during seedling growth, *Plant Physiol*, 7 (1983) 460–466.

30 Prasad M P & Munjunath K, Comparative study on biodegradation of lipid-rich wastewater using lipase producing bacterial species, *Ind J Biotech*, 10 (2011) 121–124.

31 American Public Health Association (APHA), *Standard Methods for the Examination of Dairy Products*, 14th Ed., Washington DC and applications of lipases, *Biotech Adv*, 19 (1978) 627–662.

32 Kasetsart J, Biotreatment of high fat and oil wastewater by lipase producing microorganism, *Nat Sci*, 36 (2002) 261–267.

33 Cammarota M C & Freire D M G, A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content, *Bioresource Technol*, 97 (2006) 2195–2210.

34 Demirel B, Yenigun O & Onay T T, Anaerobic treatment of dairy wastewater, *Process Biochem*, 40 (2005) 2583–2595.

35 Ning L & Hua Z M, Lipases from the genus *Penicillium*: Production, purification, characterization and applications, *J Molecul Cata B: Enzymatic*, 66 (2010) 43–54.

36 Nair J H, Lipase in raw, heated, and desiccated milk, *Ind Eng Chem*, 22 (1930) 42–45.

37 Hasan F, Ali Shah A & Hameed A, Industrial applications of microbial lipases, *Enzyme Microbial Technol*, 26 (2006) 235-251.

38 Sharma R, Chisti Y & Banerjee U C, Production, purification, characterization, and applications of lipases, Biotech Adv, 19 (2001) 627–662.

39 Gavala N, Kopsinis H, Skiadas IV, Stamatelatou K & Lyberatos G, Treatment of dairy wastewater using an upflow anaerobic sludge blanket reactor, *J Agri Eng Res*, 73 (1999) 59–63.

40 Doudoroff M & Paleroni N J, *Gram-negative aerobic rods and cocci. Genus I. Pseudomonas*, in Bergey's Manual of Determinal Line Bacteriology edited by R E. Buchnan, and N.E. Gibbons, (Williams and Wilkins, Baltimore), 1974, 217- 243.

41 Gupta R, Gupta N & Rathi P, Bacterial lipases: an overview 4. of production, purification and biotechnological properties, *Appl Microbiol Biotechnol*, 64 (2004) 763-781.

42 Kumar D & Desai K, Pollution abatement in milk dairy industry, *Curr Pharma Res*, 1 (2011) 145-152.