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**Review Article**

# Production of Lipases from Dairy Industry Wastes and its Applications

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**A B S T R A C T**

In the present era, the main pollutant derived from the industrial waste waters are organic and inorganic substances, present either in solved or in suspension, with different degree of harmfulness. The dairy waste is basically organic and slightly alkaline in nature, when discharged in to streams without treatment, result in rapid depletion of dissolved oxygen(DO) and encourage the growth of algae i.e. eutrophication (Forsberg, 1998).Due to the overuse of surfactants in dairy, the waste can become unnameable to the biological treatment. The treatment of dairy wastewater, so that it is microbiologically and chemically acceptable for use in flush and irrigation applications, is of great importance (Ibekwe *et al.*, 2003). Every Dairy industry is having particular characteristics of effluents. By the effective treatment of effluent several microbiota were identified and adapted to the wastewater physical-chemical conditions to metabolize different organic compounds in to simplest form (Mihaela Palela *et al.*, 2007). The current review focuses on utilization of some of the microorganisms involved in fast biodegradation of the organic compounds majorly lipids which have wide range of industrial applications.

## Introduction

Dairy industries are present all over the world and manufactures wide range of products like cheese, milk, butter, milk powder etc. which are involved in production of solid and liquid wastes. The dairy industry can be divided into several production sectors. Each division produces wastewater of a characteristic composition, depending on the kind of product that is produced.

The effluents from dairy industry consist of wide range of proteins, fats and possibly other additives. In the process of aerobic systems the sludge produced from the wastes of dairy industry is about 0.5 kg per kg of removed Chemical Oxygen Demand (COD) and in anaerobic systems about 0.1 kg per kg of removed Chemical Oxygen Demand (COD).

In case of dairy industry, 'lipases' are generally applied to those enzymes produced from microbes of wastes from dairy industry and increase in Free Fatty Acid (FFA) content through the hydrolysis of butterfat.

In the field of biotechnology a wide range of enzymes were commercially synthesized from screened and selected microorganism. These selected microorganisms have been characterized, optimised and purposely designed in order to produce a high-quality enzyme. These enzymes have wide industrial applications for various biological processes.

Recently in the field of Genetic engineering a branch of molecular biology, mainly conduct the experiments to tailor the genotype of a specific microorganism like *Streptomyces sps*. Hence it can produce not only the high enzyme yield, but also enzyme with desired special characteristics such as thermo stability, high temperature tolerance and stability in acidic or alkaline environment, and retaining the enzyme activity under extreme reaction conditions like heavy metals.

## Characteristics of dairy effluents

Dairy effluents contain dissolved sugars and proteins, fats, and possibly residues of additives. The key parameters are Biochemical Oxygen Demand (BOD), with an average ranging from 0.8 to 2.5 kilograms per metric ton (kg/t) of milk in the untreated effluent; Chemical Oxygen Demand (COD), which is normally about 1.5 times the BOD level; total suspended solids, at 100–1,000 milligrams per litre (mg/l); total dissolved solids: phosphorus (10–100 mg/l), and nitrogen (about 6% of the BOD level). Cream, butter, cheese, and whey production are major sources of Biological Oxygen Demand (BOD) in wastewater. The waste load equivalents of specific milk constituents are: 1 kg of milk fat = 3 kg COD; 1 kg of lactose = 1.13 kg COD; and 1 kg protein = 1.36 kg COD. Most of the solid wastes can be processed into other products and by-products.

The effluent characteristics differ from each and every dairy industry. The effective treatment of the effluent is possible only by the study of wastewater microbiota in order to identify some new active strains which are adapted to the wastewater physico-chemical conditions. This microbiota can metabolize the organic compounds, similar to those which determine the pollution of wastewaters such as starch, casein, basic carbohydrates and lactic acid (Mihaela Palela et.al; 2007).

Already there are some strains like *A.niger, Pseudomonas sps, Streptomyces sps,* which are identified and able to degrade the organic compounds easily. The microorganisms can degrade the fats/lipids present in the waste water and further isolated for the production of lipase which has wide industrial applications.

## Lipases

Lipases (EC 3.1.1.3) are mainly ester hydrolases, which catalyzes the hydrolysis of triacylglycerol to glycerol and fatty acids. These microbial lipolytic enzymcs has paved a way for many microbiologists to develop convenient and reliable methods for the detection and enumeration of lipolytic microorganisms.

Lipases are ubiquitous enzymes which are found in animals, plants, fungi and bacteria. Due to the substrate specificity and a variety of different enzymatic properties, such as broad sources, short cycle, wide pH, wide range of temperature, microbial lipase had played important role than animal and plant lipases in enzymatic theoretical research as well as practical application, including hydrolysis, esterification, trans esterification, and ester chiral synthesis.

Due to wide range of industrial applications, lipases from microorganisms are more interesting because (1) they can be produced in the high yields (2) there are many varieties of catalytic activities that can be used in many applications. (3) The genetic manipulation is easily available.

The lipases from microbial origin are heat-stable and involved in the spoilage of a various dairy products. During the culture of lipase producing bacterium the following factors should be considered i.e., i) microbial growth ii) lipase production under suitable conditions iii) detection of lipase activity of the enzyme produced

Growth conditions which affect the lipase synthesis and production are carbon source, nitrogen source, presence of metal ions such as activators and inhibitors, incubation temperature, pH, amount of inoculum used and oxygen tension. Mainly the carbon sources like olive oil, triacylglycerols, tweens, fatty acids induces the expression of lipase production.

Lipases are used to catalyse interesterification of fats and oils to produce modified glycerides unobtainable by convcntional chemical interesterification. Lipase production is also used as a marker for pathogenicity in somc medically important bacteria.

## Lipolysis

The process of Lipolysis, is the hydrolysis of lipid material to release free fatty acids (FFA) and partial glycerides, which have either detrimental or beneficial effects in the environment. In the dairy industry, with lipolysis the production of off-flavours like rancid, butyric, bitter, unclean, soapy and astringent are the major quality defect associated problems Lipolysis has been reported in a variety of stored dairy products including milk, milk powder, butter, cheese and ultra-heat-treated (UHT) milk. The process of Lipolysis in milk is resulted due to the action of either native milk lipase or lipases of microbial origin.

Lipolytic enzymes have wide range of industrial applications in various industries like food, dairy, paper, textile, leather and detergent industries, waste water treatment, production of fine chemicals, pharmaceuticals and cosmetics, synthesis of surfactants and polymers, vegetable fermentation and meat product curing. Other important application of lipases is the production of biodiesel. The biodiesel can be produced form dairy effluents by the action of lipases along with methanol and catalyst mostly Sodium hydroxide (NaOH) in a batch reactor during the process glycerine is produced as a by product after the process of trans esterification reaction.

## Applications of Lipases

Lipases occur widely in nature, but only microbial lipases are commercially significant. There are many applications for lipases which include special organic synthesis, modification of fats, flavor enhancement in food processing, resolution of racemic mixtures, and chemical analysis.

## Fat and Oleo-chemical industry

Lipases have enormous application in Oleo-chemical industry as thermal degradations can be minimized during hydrolysis, glycolysis, and also save energy. It has been reported that commercial use of *C.cylindracea* lipase in production of soap (Saxena R.K. *et al.*, 1999).

## Detergent industry

In the enzyme industry, lipases are second group of detergent enzymes. In order to assess the compatibility of lipase, it was compared with several commercially used detergents, bleaching agents to remove fat. The use of enzymes is found in all detergent formulations which are presently common in developing countries, containing enzymes. *Thermomyces sp.*, is the most important detergent lipase which is very commonly used. The *pseudomonas* spp. at alkaline pH and higher temperatures all these properties make attractive choice for detergent application (Lenting *et al.*, 1993). Therefore, a large number of *Pseudomonas species* have been studied for finding attractive and effective lipase application in different fields (Mobarak-Qamsari *et al.*, 2011).

### Polymer production

Lipases act as biocatalyst for the production of biodegradable compounds. By direct esterification of butanol 1-Butyl locate was produced and oleic acid tends to decrease the viscosity of biodiesel during winter. Trimethylolpropane esters were also similarly synthesized as lubricants. Lipases can also act as catalyst in ester syntheses and transesterification reactions in presence of organic solvent systems. Hence biodegradable polyesters and aromatic polyesters can also be produced (Linko *et al.*, 1998).

### Food processing and Flavour development

The majority of enzymes used in industry are for food processing, mainly for the modification and breakdown of biomaterials. A large number of fat clearing enzymatic lipases are produced on an industrial scale. Lipases are also utilized in dairy products for flavor development and processing other foods, like meat, vegetables, fruit, baked foods, milk product and beer (Aravindan *et al.*, 2007). The dairy industry lipases are used to modify the lengths of fatty acid chain that enhance the flavors of cheeses. A whole range of microbial lipase is prepared *M. miehei*, *A. niger*, *A.oryzae* and several others used in cheese manufacturing industry.

### Medical and Pharmaceutical sectors

Microorganisms like bacteria, yeasts, molds, and a few protozoa are known to secrete lipases for the digestion of lipid materials. Lipases from *C. Rugosa*, lowers serum cholesterol level. *S. Marcescens* have been used to synthesize lovastatin, a drug in which that lipase was widely used for the asymmetric hydrolysis of 3- phenylglycidic acid ester which is vital intermediate in the synthesis of diltiazem hydrochloride (Gurung *et al.*, 2013; Sharma & Kanwar, 2014).

### Paper and Pulp industry

Wood is the chief source of paper and pulp industry and the presence of hydrophobic components (mainly triglycerides and waxes) are helpful for production of paper. Mainly *C.rugosa* (Demuner *et al.*, 2011)was used for the production of lipase in this industry.

### Biosensors

Quantitative determination of triacylglycerol can be done by immobilisation of lipases. The process of immobilization of lipases is fast, efficient, accurate and cost effective as sensors. This application is important in the food industry, especially in fats and oils, beverages, soft drinks, pharmaceutical industries and also in clinical diagnosis (Ray, 2012). The basic concept of using lipase as biosensors is to generate glycerol from the triacylglycerol in the analytical sample and to quantify the released glycerol by a chemical or enzymatic method (Pandey *et al.*, 1999.) This method is developed for the determination of organo phosphorous pesticides with a surface acoustic wave impedance sensor by lipase hydrolysis. A biosensor based on the enzyme catalysed dissolution of biodegradable polymer films has been developed. In the polymer-enzyme system like poly (trimethylene) succinate, was used as the sensor, which was degraded by a lipase.

Potential fields of application of such a sensor system was in the detection of enzyme concentrations and the construction of disposable enzyme based immunosensors, which employ the polymer degrading enzyme as an enzyme label (Sumner *et al.,* 2001). Radiolabelled polynucleotide probes have been employed extensively for the detection of complementary nucleic acids by specific hybridization. Within the last few years, various methods have been developed using enzyme-labelled probes tends to avoid unstable and hazardous isotopes. By screening various hydrolytic enzymes to fit the special demands, fungal lipases turned out to be the most practical (Hasan *et al.,* 2006). Lipases may be immobilized onto pH/oxygen electrodes in combination with glucose oxidase, and these function as lipid biosensors and may be used in triglycerides (Ramani & Sekaran, 2012)and blood cholesterol determinations (Gandhi, 1997).

## Oil biodegradation

Biodegradation of Petroleum hydrocarbons in cold environments includes Alpine soils, due to the action of indigenous cold-adapted microorganisms which can able to degrade these contaminants. Several genotypes involved in the degradation of n-alkanes (Margesin *et al.,* 2003), aromatic hydrocarbons (Pinkart et. al., 1996), and polycyclic aromatic hydrocarbons(Khan et.al., 2001) was determined in 12 oil-contaminated have found that monitoring of soil microbial lipase activity is a valuable indicator of diesel oil biodegradation in freshly contaminated, unfertilized and fertilized soils. Various fungal species can be used to degrade oil spills in the coastal environment, which may enhance eco restoration as well as in the enzymatic oil processing in industries (Gopinath *et al.,* 1998).

## Waste and Sewage treatment

In activated sludge and other aerobic waste processes, where thin layers of fats must be continuously removed from the surface of aerated tanks to permit oxygen transport (to maintain living conditions for the biomass), this can be facilitated by use of lipases. The skimmed fat-rich liquid (Gandhi, 1997) is digested with lipases such from *C. rugosa.* Effective breakdown of solids and the clearing and prevention of fat blockage or filming in waste systems are important in many industrial operations like (i) degradation of organic debris—a commercial mixture of lipase, cellulase, protease, amylase, inorganic nutrients, wheat bran, etc. Is employed for this purpose; (ii) sewage treatment, cleaning of holding tanks, septic tanks, grease traps, etc. The first stage in the degradation and recycling of primary sewage sludge and particulate organic matter is the solubilization and enhanced hydrolysis of complex polymeric organic carbon structures associated with the anaerobic sulphidogenic environment.

## Bio diesel production

Lipase catalyzed biodiesel production was reported earlier at 2012 (Rajarshi K *et al.,*) Lipase-catalyzed trans esterification which involves two steps. Firstly it involve in hydrolysis of the ester bond and secondly on esterification with the second substrate (Fan *et al.*, 2012). Although lipases from different sources are able to catalyze the same reaction, bacterial and fungal lipases are mostly used in biodiesel production such as *A.niger*, *C.antarctica*, *C.rugosa*, *C.viscosum*, *M. miehei*, *P.cepacia*, *P.fluorescens*, *P.lipolyticum*, Rhizopusoryzae, Streptomyces sp., and *T.lanuginose*. *C.rugosa*, obtained from yeast, is the most commonly used microorganism used for the production of lipase (Sharma *et al.*, 2001). Recently, *Streptomyces sp.* was investigated as a potent lipase producing microbe for biodiesel production.

Industrial effluents from different industries may have been playing an important role in our social economy and creating serious problems. These can be solved by the isolates obtained from the treatment of the effluents of Dairy. Waste water microbiota study involved in the isolation of Lipase producing microbes which can represent an extremely versatile group of bacterial extracellular enzymes that are capable of performing a variety of important reactions, therefore helpful for future research. The understanding of structure-function relationships will enable researchers to tailor new lipases active at low temperatures for biotechnological applications. The growing demand for lipases has shifted the trend towards prospecting for novel lipases, improving the properties of existing lipases for established technical applications and producing new enzymes tailor-made for entirely new areas of application.

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