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Ultrasound assisted enzymatic pre-treatment of high fat content dairy wastewater

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ABSTRACT

This paper illustrates the application of ultrasound in a dairy waste water treatment for the removal of fat using enzyme as a catalyst. Lipase Z was used to perform the enzymatic pre-hydrolysis of a synthetic dairy wastewater containing around 2000 mg/L of fat content coupled with ultrasound irradiation. Different process parameters like effect of enzyme loading, temperature, ultrasound power, frequency, duty cycle and speed of agitation are optimized. The maximum hydrolysis of 78% is achieved at 0.2% enzyme loading (w/v), 30 °C temperature, 165 W of ultrasonication power at 25 kHz and 66% duty cycle. It was observed that the enzymatic pre-hydrolysis under the influence of ultrasound drastically reduces the reaction time from 24 h to 40 min as compared to conventional stirring with improved yield.

1. Introduction

Even being a renewable source, the world's supply of fresh water resources is steadily exhausting propagating global catastrophe. In recent decades, the rate at which humans worldwide are pumping the underground stores of water for anthropogenic activities has taken toll on the water resources. Apart from this, the effluent of domestic and industrial usage are still dumped in water bodies and therefore recently replenishing is gaining wide consideration leading to development of various schemes to revert less hazardous water to its sources [1]. The reuse of waste water is one of the alternatives to solve the problem of water crisis. The amount waste water generated from industries varies depending upon the water utilization as well as the type of industry. The dairy industry is one of the major industries all the over the world. The various improvements in the field of veterinary science led to continuous rise in the milk production per head of cattle and substantial increase in the milk and milk products demand in many countries [2].

In India, dairy industry is one of the economy boosting sectors, but its pollution potential cannot go overlooked [3]. It generates 6–10 L of effluents/L of milk. It's not only increasing pollution with respect to the volume of effluent generated but also in terms of its properties [2]. The dairy wastewater is basically composed of carbohydrates (lactose), proteins and fats. Thus, it is characterized by large amount of organic molecules, high fats, oils and grease (FOG) content along with milk solids, detergents, sanitizers, milk wastes etc. These FOGs can cause serious issues of organic loading on local sewage drainage system [4,5]. The dairy wastewater includes wash water from cleaning bulk tanks, milk pipelines, milking units, miscellaneous equipments, washing milk silos or operational malfunctions [3,4,6]. The dairy waste water contains large amount of organic products of which the FOGs in a dairy wastewater poses a major threat to the environment. They have high molecular weight and low biodegradability coefficient and thus subsequently outlined by high biological oxygen demand (BOD) and chemical oxygen demand (COD), creating land and water pollution, contaminating ground water. The FOGs tend to degrade slowly and are present permanently in some kind of waste and they form a film over the surfaces of water bodies preventing the proper diffusion of oxygen leading to the death of aquatic flora and fauna, eutrophication, thus hampering eco-system [5,7]. In aerobic reactors, the formation of such films and greasy layers hinders the flocculation and sedimentation process of the biomass which intern interrupts pumping and subsequently the gas transfer which is much needed for the biological degradation [8].

To lessen these outburst, the biological treatment for the dairy wastewater is appeared to be most promising techniques and usually aerobic treatment processes like trickling filters, activated sludge processes, aerated lagoon and sequencing batch reactor (SBR) are used [3,9]. However, recently anaerobic treatment processes have been found to be more attractive over traditional aerobic system because of valuable biogas production, less biomass and sludge generation, higher organic loading application, no need of aeration devices, less area required and much less amount of energy consumption than the aerobic counterpart [10,11].

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Moreover, the large amount of FOGs contents were found to be repressive for anaerobic treatment due to flotation of sludge deposition of fat scum layer on reactor surface [11]. Also solidification of fats at low temperature may lead to clogging and even develop obnoxious odor. Thus, the decrease in performance of the process can be blamed to the low hydrolysis in the anaerobic reactor. Thus, evoking the need to exercise pre-treatment systems to remove oils and grease from wastewater prior to the main biological treatment process [7].

Hydrolysis and dissolution of fats in pre-treatment processes, boosts biological degradation of fatty dairy wastewater, thus accelerating the process and reducing the time consumed [5]. The physico-chemical pre-treatment techniques used did not render a good COD removal, the reagents used are costly and these methods required harsh reaction conditions with subsequent generation of problematic sludge [5,12].

Hence, biochemical techniques like using specific enzymes-lipases acquired consideration owing to the stringent environmental regulations, clean and friendly approach of enzymes [7], mild reaction conditions, high specificity and easy availability. Enzymes are known to act as versatile catalyst with enhanced application in the field of biotechnology. Their characteristics present them as an attractive alternative for waste/pollutant treatment over conventional treatment [13].

Lipases break down triglycerides to free fatty acids and glycerol in the presence of oil/water interface [14]. These enzymes showed promising appliances in degradation of FOGs in dairy wastewater from dairies, slaughterhouses, pet food industry [10,15].

In the present study, Lipase Z was used as it shows positional specificity against three ester linkage of triglyceride giving high hydrolysis rate. In addition, because lipase Z catalyzes the triglyceride hydrolysis at low temperature around 30 °C this enzymatic process does not cause undesirable reaction of fatty acids. Also, the lower temperature reaction by the enzymes brings substantial savings of energy cost. However, the main concern with the enzyme pre-treatment is the higher time required to for the hydrolysis.

Further if the enzyme catalyzed pre-treatment is coupled along with ultrasonic irradiation it resulted in significant degree of process intensification due to phenomenon of mass transfer by cavitation [16]. Irradiation with ultrasound waves leads to cavitation i.e. the formation, growth and implosive collapses of bubbles causes release of energy which results in increase of mass transfer rate. Apart from this, it is also accountable for the thermal and mechanical effect due to cavitation which enhances the mass transfer further [17]. Thus, it is attractive than the conventional reaction because of improved mass transfer rate and less reaction time required. However the application of ultrasound for the lipase catalyzed pre-treatment of dairy waste water treatment has not been explored earlier.

Hence, the aim of this work is to investigate the enzyme catalyzed hydrolysis of fatty dairy wastewater carried out prior to the anaerobic digestion step, to reduce the earlier mentioned operational limitations with the assistance of ultra-sonication to decrease the long reaction duration required and thereby, increasing the efficiency of reaction.

2. Materials and methods

2.1. Materials

2.1.1. Enzymes

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

2.1.2. Wastewater

Synthetic dairy wastewater was prepared by diluting different proportions of milk and deionized water to acquire various fat concentrations. The amount of fat content varied in order to reach approximately around 2000, 4000, 6000 and 8000 mg/l much higher than the normal fat content of dairy wastewater which ranges from 200 to 1200 mg/l. The milk was bought from a local dairy, Sharma Dairy, Mumbai. The proportion of milk:water (1:8) was used for the standardization study (2000 mg/l). This prepared wastewater was used for the enzymatic hydrolysis integrated with ultrasound and for studying the characteristics of dairy wastewater. When not in used, it 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. Chemical oxygen demand (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

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

2.2.3. Titrimetric analysis

The acid value of the reaction was evaluated by titrating it with 0.02N aqueous KOH solutions. The acid value is determined using following equation.

$$\text{Acid value} = \frac{56.1 \times N \times (B - S)}{W} \quad (1)$$

where N is Normality of alkali N, B and S are blank and sample values in ml, W is the weight of fat in sample. Further, the % hydrolysis is obtained by ratio of acid value and saponification value of fat.

2.3. Experimental method

2.3.1. Conventional stirring

Conventional batch experiment was performed in a 150 mL flat bottom baffled glass reactor equipped with a six-bladed turbine glass impeller. Around 100 mL of prepared wastewater was fed in the reactor along with an enzyme loading of 0.2% (w/v) and sodium chloride as an emulsifying agent (100 mmol). This assembly was put in a water bath maintained at 30 °C. The reaction mixture was agitated with a mechanical stirrer at a speed of 200 rpm by means of an electric motor equipped with speed controlling device. The reactions were carried out for a maximum period of 24 h. At specific time intervals, a small sample (2 mL) was withdrawn from the reactor and transferred to conical flask. 10 mL of a 50:50 v/v

Table 1
Characteristics of the dairy wastewater used in this study.

Parameters	Values
pH	6–6.5
Density (g/cm^3)	1.005
Free fatty acids (%)	0.28
Lipids/FOG (mg/L)	2000
COD (ppm)	42,000
Acidity (mg.NaOH/L)	0.4