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Biological treatment of synthetic dairy wastewater in FBBR

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The dairy industry produces large amounts of wastewater that have higher potential to pollute environment if not properly treated. It is characterized by high levels of BOD, COD, organic and inorganic nutrients. This study focuses on the aerobic treatment of synthetic dairy wastewater in fluidized bed bioreactor using low density bio carrier wood particle at optimum conditions of temperature and pH and also the identification of the microbes which are effective for the degradation of the dairy waste. The microbial isolates were tentatively identified to be *Lactobacillus* and *Bacillus*. It is observed that with the increase in influent COD there is a decrease in the percentage COD reduction of the effluent The COD reduction at optimum temperature 30°C and pH 7 is found to be 84% and BOD removal to be 75% efficient for an incubation period of 120 h.

Keywords: Dairy wastewater, COD, BOD, aerobic treatment .

Introduction

The dairy industry is a major food processing sector that processes items such as milk, butter, milk powder, and cheese and also produces large amount of solid and liquid waste[1]. It is usually characterized by a high demand for (BOD) and (COD)[2]. High organic load containing wastewater from the dairy industry when discharged into water reservoirs results in the depletion of dissolved oxygen level and becomes the breeding ground for insect and flies and cause dangerous diseases like malaria, yellow fever, and dengue[3]. Different methods are used in the treatment of dairy waste water such as wetland treatment, physico-chemical and biological treatment. One of the most promising methods for treatment dairy wastewater is the biological approach. Fluidized bed bioreactor is shown to work better than other type of bioreactor[4]. The fluidized bed bioreactors has an exceptional performance due to the cell immobilization of solid materials which reduces the treatment time, the reactor volume is extremely small, the lack of biomass clogging and the removal of contaminants even at lower concentrations[5]. Hence the present research was intended to treat synthetic dairy wastewater in a fabricated FBBR using mixed culture. It also aims to study the physicochemical characteristics of synthetic dairy wastewater, isolate, screen and identify the microbes that can efficiently treat synthetic dairy wastewater and also perform batch biodegradation experiments in shake flask

Materials and methods

Materials:

The chemicals used for the study were of analytical grade, whereas the glucose and inorganic salts used in the preparation of the microbial growth media were of reagent grade. All other chemicals were being obtained from Merck®, India and HIMEDIA®, India.

Isolation and identification of the bacterial strains:

The dairy wastewater and soil samples into which the wastewater was discharged was collected from dairy plant, Rourkela in Sundargarh district, Odisha. The samples were collected in sterile bottles and stored at 4°C until the analysis was carried out. The samples were plated on specific nutrient agar plates and incubated overnight at 37°C. The prominent and well isolated potential bacterial colonies capable of growing and reducing COD in the effluent were maintained in nutrient agar slants at 4°C for future studies. A total of 4 isolates were collected and designated as OS1, OS2, OS3, OS4. These microbial inoculum were prepared and used for the biodegradation studies. Identification of the isolates was based on morphological observation and bio-

chemical characterization. The tests involved were motility, cell shape, colony structure, oxygen demand, nitrate reduction, catalase, indole production, oxidase, dextrose fermentation, sucrose fermentation, gelatin liquefaction, lactose fermentation, citrate utilization, etc. Bergey's manual of determinative of bacteriology was used as a reference for the isolates found.

Preparation and characteristics of synthetic dairy wastewater:

The synthetic dairy wastewater was prepared by mixing NH_4Cl 280 mg, KH_2PO_4 200 mg, $MgSO_4.7H_2O$ 8 mg, $CaCl_2.H_2O$ 5 mg, $NaHCO_3$ 350 mg , glucose 100 mg, milk powder 1500 mg in 1 L of distilled water. Synthetic dairy wastewater of given composition of about 4–5 L is prepared and autoclaved so that contamination doesn't exist. The characteristics of synthetic wastewater such as pH, COD, BOD, TDS are studied with standard methods (APHA, 1992).

Analytical methods:

Biomass concentration was determined by spectrophotometrically at 600 nm in UV-Vis spectrophotometer. The pH was determined by direct pH meter. The turbidity of the sample were measured by Nephlometric method in Century turbidity meter CTD401. Biological oxygen demand (BOD) was analyzed by Winkler's method and chemical oxygen demand was analyzed by dichromate method.

Batch biodegradation studies:

In order to study the biodegradation efficiency of the each isolates individually, batch experiments were carried out in 250 ml Erlenmeyer flasks under shaking at 120 rpm. Each flask containing 100 ml of synthetic dairy sample were inoculated aseptically with each isolates and incubated at 30°C. For the purpose of increasing the pollutant removal a combination of the four selected bacterial strain named OS1, OS2, OS3, OS4 were also studied. The samples were analysed for growth and COD reduction.

Optimisation studies:

Growth and biodegradation behaviour of the microorganism depends on the physiological parameters. Microorganism develops within a range of physiological parameters, but at the particular conditions of the physiological parameters optimum growth is achieved (Palkova, 2004). In this study the physiological parameters such as pH, temperature, inoculum size were used to optimize the maximum degradation of the synthetic dairy effluent. The inoculum size were varied between 2–8%, pH in the range 5-8 and temperature from 25–35°C.

Experimental setup:

A fabricated fluidized bed reactor is used to study the biodegradation characteristics of synthetic dairy wastewater by immobilized mix culture of the bacterial strain on low density biocarrier wood strips. The fluidized bed assembly is divided into three sections, namely the test section, section of the gas-liquid disengagement and section of the gas-liquid distributor. Fig. 1 shows the photographic representation of the experimental setup used for the study. The main component of the fluidized bed reactor is the test section where fluidization and degradation takes place the maximum. A vertical cylinder Plexiglas column having of 4.9 cm internal diameter, column height 60 cm and there is protrude of 8 cm within the disengagement section. The capacity of the fluidizer is 1.13 L. The gas-liquid distributor is placed at the bottom of the test section and is designed such that the liquid and gas mixture enters the test section in a relatively uniform manner. A 4.3 cm diameter cross-type air sparger with 8, 1 mm holes was attached below the distributor plate and screwed to the main body of the distributor for the generation of fine bubbles evenly distributed along the column cross-section of the fluidizer. Visually, the distributor arrangement provides a uniform flow of liquid and gas to the text section, with fine gas bubbles. The gas and liquid streams were merged and passed through the wired mesh in the gas-liquid distributor section. The gas-liquid disengagement portion of volume 3.9 L is at the top of the fluidizing segment, allowing gas to escape and liquid to flow at the bottom of this section through the outlet of 1.27 cm internal diameter. The synthetic dairy wastewater was pumped to the fluidized bed through a fractional horsepower pump (FHP) using water Rotameter to calculate the water flow rate and circulated with the circulating system. The air was pumped via the air sparger from an air compressor. The air compressor was 0.037 kw, 1440 rpm with a maximum air discharge capacity of 15 lpm at 2 bar pressure. The air flow rate was measured using a needle valve rotameter with the range 0–2 lpm.

The biocarrier used was low density particles made from wood strips (Fig. 2). The wood particles were in cubical shape

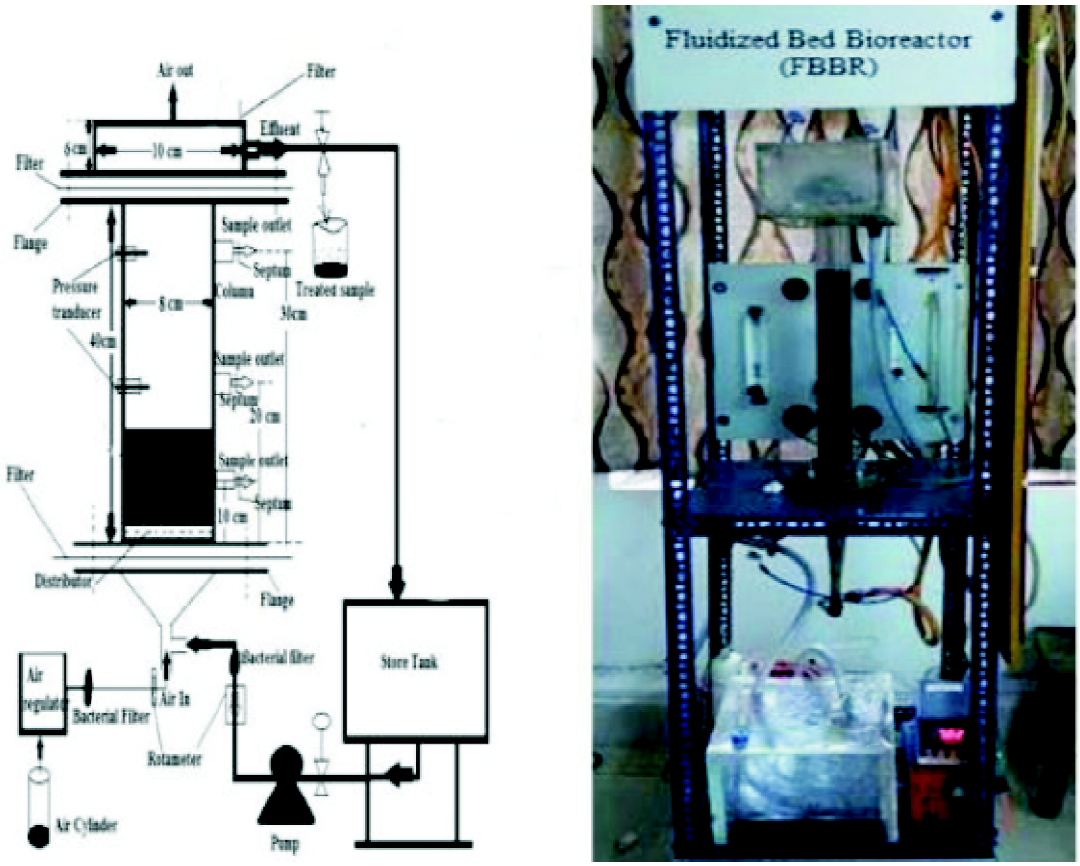

Fig. 1. Experimental setup.

Fig. 2. Low density wood particle used as biocarrier.

furnished from carpentry shop with a regular size of 7 mm. The density of wood particles was measured using the water displacement procedure. As water is having higher density than that of wood particle, these were then held in water for 4 days to increase the density of wood particle nearer to that of density of water i.e. 997 kg/m^3. After few days the density of wood particle increased by 1111 kg/m^3 as a result being having density higher than that of water, now the solid particles can be fluidised

Results and discussion

Isolation, characterization and identification of the bacterial strains:

The study focuses on the identification of the isolated microorganisms and their enhancement in biodegradation and the treatment of the synthetic dairy wastewater by immobilization in the bioreactor. In this experiment a total of 4

strains were isolated and identified. These four strains were designated as OS1, OS2, OS3, OS4 (Fig. 3). Colony characteristics and biochemical characteristics of the four isolates are described in Tables 1 and 2. By comparing the morophlgical and biochemical characteristics of the isolates and comparing it with those mentioned by Vishakha *et al*. (2013), Rajeshkumar *et al.* (2003) and with Bergey's manual of systematic bacteriology the isolate OS1 and OS3 were tentatively identified to genus *Lactobacillus* and the isolates OS2 and OS4 were identified tentatively to genus *Bacillus.*

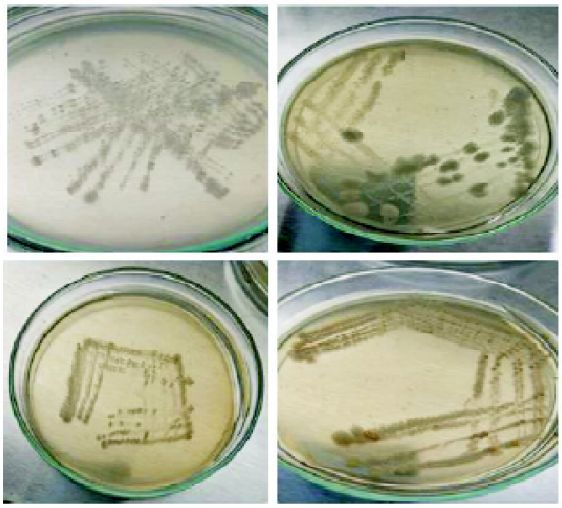

Fig. 3. Microbial isolates OS1, OS2, OS3, OS4.

Characterisics of synthetic dairy wastewater:

The synthetic dairy wastewater was prepared as per the composition given in Section *Characterisics of synthetic dairy wastewater* and sterilized by autoclaving. Before the treatment, the synthetic dairy wastewater was characterized by milky appearance, and there was change in colour after treatment. The change in colour is because of the fact that the organic matter is decomposed by the microorganism[6]. The characteristics of the synthetic dairy wastewater is shown in Table 3.

Batch biodegradation studies:

Potential strains are decided on the fact that the bacterial strain have the capability of growing and also reducing the COD in the raw effluent[5]. So the four strains OS1, OS2, OS3 and OS4 were subjected for the ability to grow and decrease COD. Since all the strains have comparable ability to reduce COD a mixed culture of *Lactobacilus* and *Bacillus* was prepared and compared with the single isolates. From the Fig. 4 it is observed that mix culture was efficient in degrading synthetic wastewater, expressed by decrease in COD reduction by 72% which was higher as compared to the single strains.

Table 1. Colony and morphological characteristics of microbial isolates

Bacterial isolate	Shape	Colour	Margin	Elevation	Opacity	Consistency
OS1	Circular	Whitish	Regular	Flat	Opaque	Moist
OS2	Round	Dirty white	Regular	Slight convex	Opaque	Moist
OS3	Round	Whitish	Regular	Flat	Transparent	Moist
OS4	Circular	Dirty white	Regular	Slight convex	Opaque	Moist

Table 2. Biochemical characteristics of microbial isolates

Sl. no.	Test	OSI strain	OS2 strain	OS3 strain	OS4 strain
1.	Gram character	+	+	+	+
2.	Motility	+	+	+	+
3.	Catalase	+	+	+	+
4.	Indole	–	–	–	–
5.	Gelatinase	–	–	–	–
6.	Urease	–	–	–	+
7.	H_2S production	–	–	–	–
8.	Starch hydrolysis	+	–	+	+
9.	Citrate	–	–	–	–
10.	Lactose	+	+	+	+
11.	Sucrose	+	+	+	+
12.	Dextrose	+	+	+	+
13.	MR Test	+	–	+	+

Table 3. characteristics of synthetic dairy wastewater

Sl. No.	Parameter	Observation
1.	pH	6–7
2.	COD	1310 mg/L
3.	BOD	600–700 mg/L
4.	Turbidity	253 NTU

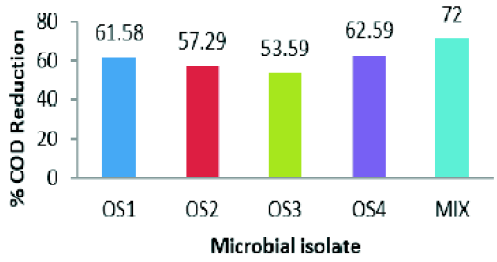

Fig. 4. Comparison of reduction in COD for mix culture and single isolates.

Effect of temperature on growth of mixed culture:

Fig. 5 shows the growth of the mix culture of bacteria at different temperature. High growth rate was observed at temperature 30 and 35°C and reduced growth rate at 20 and 40°C. Since *Lactobacillus* and *Bacillus* bacteria belong to mesophile group (20–45°C) optimal growth take place here. However improved growth rate is observed at 30°C because of the increased enzyme activity resulting in increased growth rate.

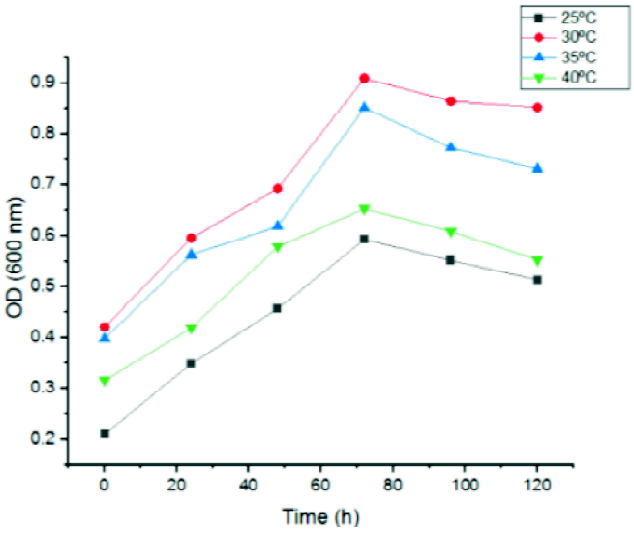

Fig. 5. Growth profile of mix culture at various temperatures.

Effect of pH on growth of mixed culture:

The growth curve of the mixed culture at different pH at 30°C was obtained. From the Fig. 6 it can be concluded that maximum growth is observed at pH 7 as compared to other pH. As per Annadurai *et al*. (1999) studies, at high pH or low

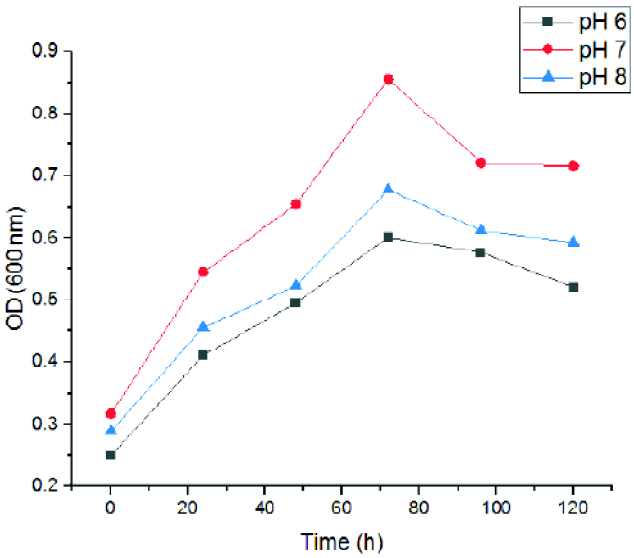

Fig. 6. Effect of pH on growth of mixed culture.

pH, acids or bases have the chances of entering into the cells easily, because in that stage the cells tend to be in undissociated forms and this cannot be prevented by the electrostatic forces. As a result the substance that are permeated alter the pH internally and decrease the microbial activity.

Effect of inoculum size:

Effect of inoculum size is a important parameter for the maximum degradation. Fig. 7 shows the effect of inoculum size in the percentage of COD removal. It can be concluded

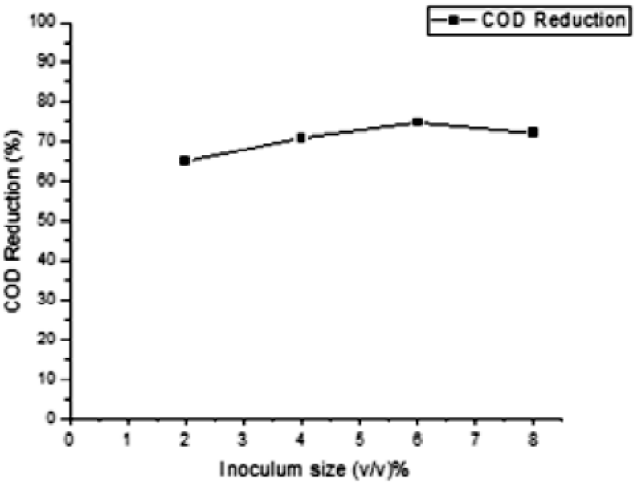

Fig. 7. Variation of % COD reduction with inoculum size.

that the rate of COD removal increases with increase in inoculum size and maximum removal rate is observed for 6% (v/v) up to a COD reduction of 74.8%. Hence for further study inoculum size 6% (v/v) was used as there was a decrease in COD reduction for 8%.

Biodegradation of synthetic dairy wastewater by immobilised cells in FBBR:

From Figs. 8 and 9 it shows that there is decrease in the COD and BOD values using the mixed culture of *Lactobacillius* and *Bacillus*. After 120 h of incubation, the COD values decreased from 1310 mg/L to 200 mg/L respectively,

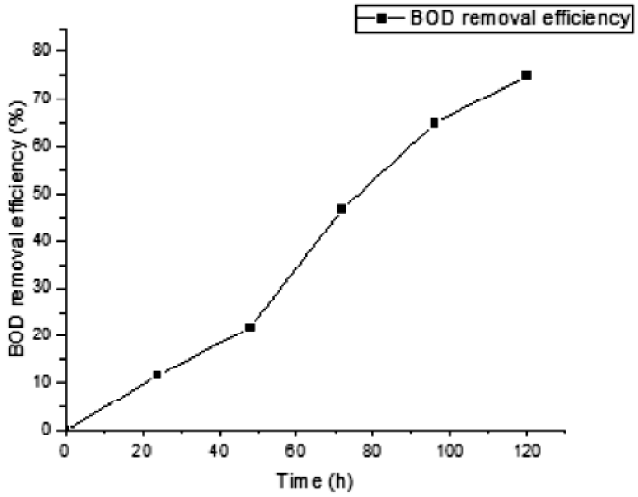

Fig. 8. Variation of BOD removal efficiency.

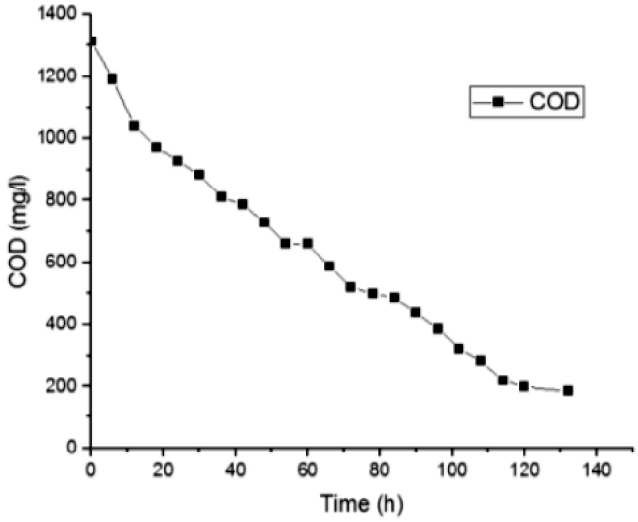

Fig. 9. Variation of COD with time for dairy concentration of 1500 mg/L.

which shows the average COD removal efficiency to be 84.7% and the BOD removal efficiency increased upto 75%. Studies conducted by Asha and Elakkiya[8] (2014), using a fixed film fixed bed bioreactor using synthetic dairy wastewater with initial COD loading of 2072 mg/L showed an average COD reduction of 71%. In the previous studies carried out by Sammaiah *et al.* (1991), with the help of upflow anaerobic filter process, it was possible to reduce COD of the dairy waste water with a average value of 77.5% with a residence time of 25 days. Arumugan and Sabarethinam[9] studied the selection of suitable support material in fluidised bed reactor from Teflon, ceramic and glass. COD reduction with glass as support material was upto 71%. In this study using wood particles as biocarrier showed a COD reduction of 84.7% which is better as compared to glass on the fact that wood being economical and is considered to be organic and easily available. Further experiment is carried out for increased concentration of dairy wastewater to 2500 mg/L. From the Fig. 10 it can be inferred that as the concentration of dairy product increased there is a decrease in the percentage of COD reduction to 79.4%. It can be associated that the COD removal percentage is decreased as the influent COD concentration is increased.

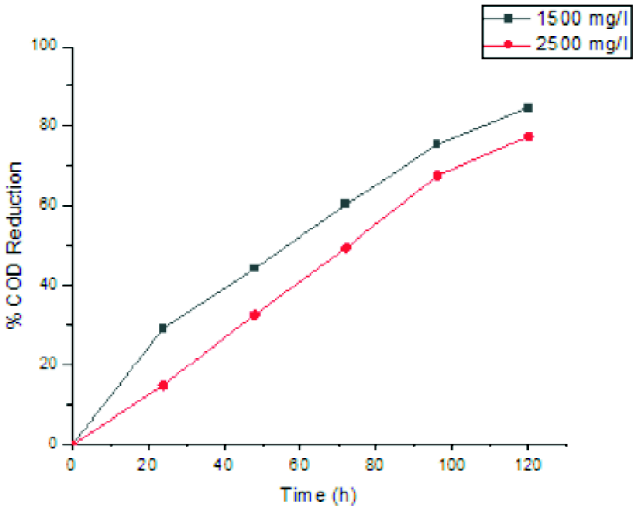

Fig. 10. Percentage reduction in COD for different concentration of dairy product.

Conclusions

Batch biodegradation study of synthetic dairy wastewater by mixed culture of *Lactobacillus* and *Bacillus* in fluidised

bed bioreactor using low density wood particles showed better degradation efficiency with a COD reduction of 84% and BOD reduction of 75%. Hence this study shows a viable solution to the treatment of dairy wastewater.

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