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Treatment of dairy wastewater containing high amount of fats and oils using a yeast-bioreactor system under batch, fed-batch and continuous operation

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ABSTRACT

This study evaluated the potential of the biosurfactant-producing yeast *Candida bombicola* in treating wastewater containing fats and oils from a dairy industry in a laboratory scale bioreactor. The dairy wastewater contains high chemical oxygen demand (COD) (2,480 mg/L) and fats and oils (407 mg/L) and was supplemented with sugarcane molasses (1% w/v) and yeast extract (0.1% w/v) to support the growing yeast in batch, fed-batch, and continuous operations. The yeast was able to remove fats and oils completely (more than 95% COD removal) under batch and continuous operation. The study suggested that wastewater containing high fats and oils can be efficiently treated using *C. bombicola*.

Keywords: Dairy wastewater; *Candida bombicola*; Fats and oils removal; Yeast bioreactor system; Sophorolipids

1. Introduction

Fats and oils are essentially triglycerides consisting of long-chain fatty acids attached, as esters, to glycerol [1]. These fats and oils when present in the wastewater create several operational problems. During aerobic treatment, they block oxygen transfer process in biological degradation, while in anaerobic process, they may solidify at lower temperatures and thus cause clogging and unpleasant odors [2]. Moreover, the long chain-fatty acids (hydrolytic product of these fats and oils) are toxic to various microorganisms [3]. High fats and oils containing wastewaters are generally discharged by the food industries, such as dairy industry [4,5].

In dairy industry, high fats and oils containing wastewater is generated in large amount from different operations, such as cleaning, sanitization, floor washing, etc. [6]. Thus, it is essential to remove fats and oils from dairy wastewater prior to biological treatment. Physicochemical methods for removal of fats and oils are costly and often exhibit lower efficiency [3,7]. For example, dissolved air floatation (at a gage pressure of 65 lb/in^2 and pressurization time of 3 min) provides on an average <80% removal of fats and oils [8]. On the other hand, the use of hydrolytic enzymes has been shown to be more effective but high production cost limits their use on a large scale [9–11]. For example, the production costs of lipase (a common hydrolytic enzyme) from *Penicillium restrictum* under submerged and solid-state fermentation are reported to be US$41.55/L and US$130.85/L, respectively [12].

Efficient use of surfactants for fats and oils removal from wool-scouring wastewater and high-strength pet food wastewater has been reported in the literature [8,13]. The use of chemical surfactants is not eco-friendly for fats and oils removal, whereas the use

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of biological surfactants is not cost-effective. In this context, *in situ* production of biosurfactants for the solubilization of fats and oils coupled to wastewater treatment using microorganisms is a better approach. Therefore, the objective of the present work was to study the treatment of high fats and oils containing real dairy wastewater by using a non-pathogenic and biosurfactant-producing yeast *Candida bombicola* under different modes of operation employing a bioreactor.

2. Materials and methods

2.1. Microorganism and maintenance

The yeast *Starmerella bombicola* NRRL Y-17069 (equivalent strain of *C. bombicola* ATCC 22214) was kindly provided by Agricultural Research Service (ARS-Culture Collection), USDA, Peoria, USA. The yeast strain was grown and maintained according to the ARS-Culture Collection's instructions. In brief, the strain was cultivated for 48 h at 30˚C incubation on agar slants containing (g/L): glucose, 10; yeast extract, 3; peptone, 5; and agar, 20 (GYP-agar). The yeast was sub-cultured at regular intervals (in every four weeks) and maintained at 4˚C in a refrigerator.

2.2. Chemicals and reagents

Sugarcane molasses used in the study was purchased from local market in Guwahati, India. All other chemicals and solvents used in this study were of analytical grade and supplied by either Hi-Media Pvt. Ltd., India, or Merck India Ltd.

2.3. Source of dairy wastewater and its characterization

Dairy wastewater used in this study was collected from a local dairy in Guwahati, India. The collected wastewater was stored in a refrigerator at 4˚C until further use. The dairy wastewater was characterized for its pH, total fats and oils content, solids, carbohydrate content, 5-day biochemical oxygen demand (BOD) (BOD_5), and total soluble chemical oxygen demand (COD) as per the standard methods [14].

2.4. Dairy wastewater treatment experiments

Experiments to study the treatment of dairy wastewater were carried out using a 3-L bioreactor (Applikon, the Netherlands) with a working volume of 1 L. Schematic of the bioreactor setup used in this study is shown in Fig. 1. All experiments with the bioreactor were carried out under non-sterile condition at 30˚C. The initial pH of the wastewater was neither adjusted nor controlled during the experiments. The wastewater was supplemented with a small amount of sugarcane molasses sugar (1% w/v) and yeast extract (0.1% w/v) to support the yeast growth. The reactor contents were agitated at 300 rpm and aerated at 1.5 L/min throughout the experiments following inoculation with a 5% (v/v) of the seed culture [15,16]. The biomass concentration in the seed culture was 2–3 g/L.

Wastewater treatment was first examined under batch mode followed by fed-batch and continuous modes. Following the initial batch experiment for 5 d, fed-batch experiment was carried out with a wastewater feed rate based on the specific growth rate and yeast biomass yield. For continuous experiment, the yeast-bioreactor system was initially operated under batch mode for up to 72 h and then shifted to continuous mode with a hydraulic retention time (HRT) of 40 h which corresponded to a dilution rate of 0.025 1/h for up to 9 d.

During all these experiments, samples (about 10 mL) were withdrawn from the bioreactor at constant time intervals and analyzed for fats and oils, yeast biomass, sophorolipids, total carbohydrate concentration, and COD.

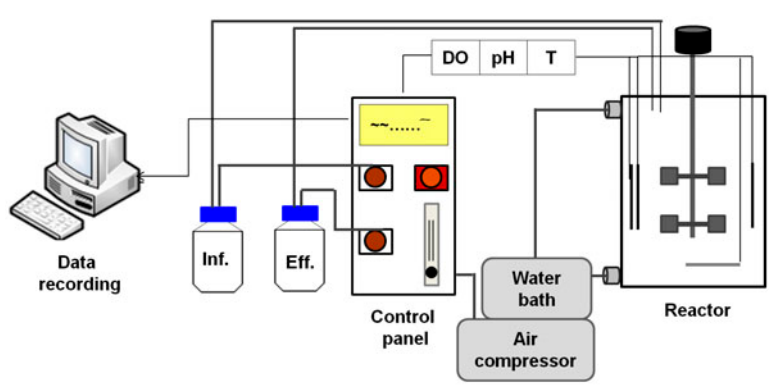

Fig. 1. Schematic of the bioreactor system used in this study.

Table 1
Characteristics of the dairy wastewater used in this study

Parameter	Value
pH	5.94
Total solids, mg/L	3,600
Suspended solids, mg/L	350
Total fats and oils content, mg/L	407
Total carbohydrates, g/L	3.0
BOD_5, mg/L	740
COD, mg/L	2,480
Color	Dark gray
Smell	Pungent

2.5. Analytical methods

Samples (without pH adjustment) were extracted twice with an equal volume of n-hexane, and the organic layer containing the fats and oils was separated, vacuum-dried at 40˚C; residue obtained after gravimetric analysis was taken as the total fats and oils [15,16].

Yeast biomass and sophorolipids concentration in the samples were estimated by cell dry weight method and gravimetrically after extraction with ethyl acetate, respectively [17]. Total carbohydrate concentration in the samples was analyzed by the Anthrone method [17,18].

3. Results and discussion

3.1. Characteristics of the dairy wastewater

Table 1 presents the dairy wastewater characteristics, which reveals that it contains a high BOD and COD. The high COD is mainly due to the presence of dissolved milk proteins, sugars, and fats and oils in the wastewater. Total fats and oils content in the wastewater was found to be ~400 mg/L, which is well within the range (60–754 mg/L) of other reported data for dairy wastewater [19]. Initial pH of the dairy wastewater is slightly acidic, that is, near 6.0, which is, however, well suited for the growth of the yeast *C. bombicola*. These characterization results are in agreement with those reported in the literature for dairy wastewater [6,20,21].

3.2. Wastewater treatment under batch operation

Fig. 2 shows the results of dairy wastewater treatment using *C. bombicola* in the batch-operated bioreactor. Complete removal of fats and oils as well as 96% COD removal was observed within 72 h of its operation. The maximum biomass concentration was attained at 66 h of the batch reactor operation. The complete removal of fats and oils is attributed to the sophorolipids produced by the yeast, which has a very low critical micelle concentration of 30 mg/L [15,16]. As a biosurfactant, sophorolipids are involved in solubilization of fats and oils in the aqueous phase, thereby enabling their better utilization and wastewater COD reduction by the yeast. The sophorolipid produced in the batch reactor was within the range 1–2 g/L (data not shown). The yield of sophorolipids in this study is much lower than those reported by Daverey et al. [16] using real dairy wastewater, which is attributed to the high levels of sugars, such as glucose, lactose, or sucrose (10%) and soybean oil (10%) used mainly for its production rather than for treating dairy wastewater.

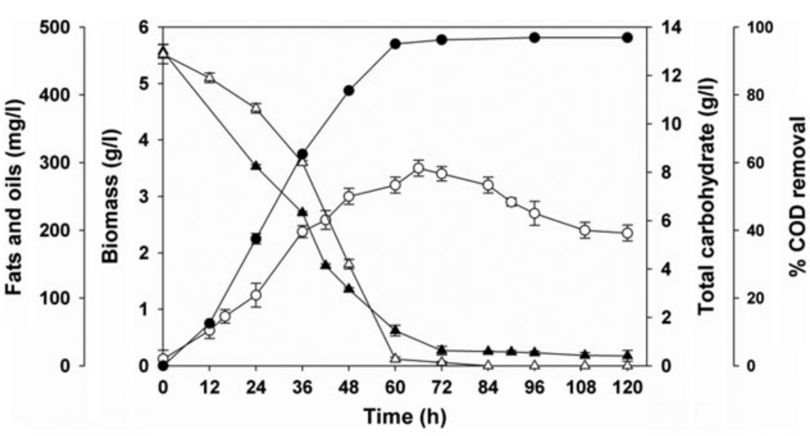

Fig. 2. Results of dairy wastewater treatment using the yeast-bioreactor system operated under batch mode (▬○▬ Yeast biomass, ▬▲▬ Total carbohydrate, ▬△▬ Fat, ▬●▬ % COD removal).

Biosurfactants produced by *Planococcus citreus* and *Pantoea agglomerans* have been shown to remove 76 and 70% COD of oily soap industry effluents in batch operation [22]. Recently, a study on the potential of exopolysaccharide isolated from *Azotobacter indicus* in the pretreatment of dairy wastewater has been reported in the literature with a total COD reduction value of less than 65% [23]. Compared to these reports, this study showed excellent results in terms of COD removal and fats and oils utilization by *C. bombicola* under batch mode of operation.

Biokinetic constants μ and $Y_{X/S}$ were estimated from the results of the batch experiment and using the following Eqs. (1) and (2), respectively:

$$\mu = \frac{1}{X}\frac{dX}{dt} \tag{1}$$

$$Y_{X/S} = \frac{X_m - X_0}{S_0 - S_m} \tag{2}$$

In Eqs. (1) or (2), X_m represents maximum cell concentration (g/L) at time (t), X_0 is the initial cell concentration (g/L) at initial time ($t = 0$), S_m is the total substrate concentration (g/L) at time (t) and S_0 the total substrate concentration (g/L) at initial time ($t = 0$).

These values were estimated to be 0.055 1/h and 0.39 for μ and $Y_{X/S}$, respectively, in the batch operation, which are found to be slightly lower than those obtained using synthetic dairy wastewater (μ, 0.072 1/h and $Y_{X/S}$, 0.67) [15]. This difference in the biokinetic parameter values can be easily attributed to the composition of the real dairy wastewater, which is highly complex compared with that of a synthetic dairy wastewater.

3.3. Wastewater treatment under fed-batch operation

For fed-batch experiments, the yeast-bioreactor system was initially operated under batch for 48 h followed by continuous wastewater feeding. The wastewater feed rate was calculated as per the following equation [24]:

$$F = \frac{\mu X_0 V_0 e^{\mu t}}{Y_{X/s} S_0} \tag{3}$$

where, F is the wastewater feed rate (L/h), μ is the specific growth rate (1/h) of the yeast; X_0 is the yeast biomass concentration at the end of batch operation (g/L); V_0 is the volume of wastewater in the bioreactor (L) at the end of batch; $Y_{X/S}$ is the yeast biomass yield and S_0 is the total carbohydrate taken as input to the wastewater (g/L).

Fig. 3 shows the results of fats and oils removal from the wastewater obtained in the fed-batch experiment. These results are better compared to that obtained in the batch experiment as complete utilization of fats and oils within 72 h and 97% COD removal efficiency at the end of 96 h were recorded. The concentration of sophorolipids (1–2 g/L) produced by the yeast under fed-batch mode was, however, the same as found in the batch experiments. Besides, the amount of wastewater treated in the fed-batch experiment was twice the amount treated in the batch experiment.

During the initial batch operation (0–48 h), concentrations of fats and oils, and carbohydrate quickly decreased to 165 mg/L and 2.5 g/L, respectively, and a COD removal efficiency of about 80% was observed after 48 h. In the second stage (48–60 h), fresh

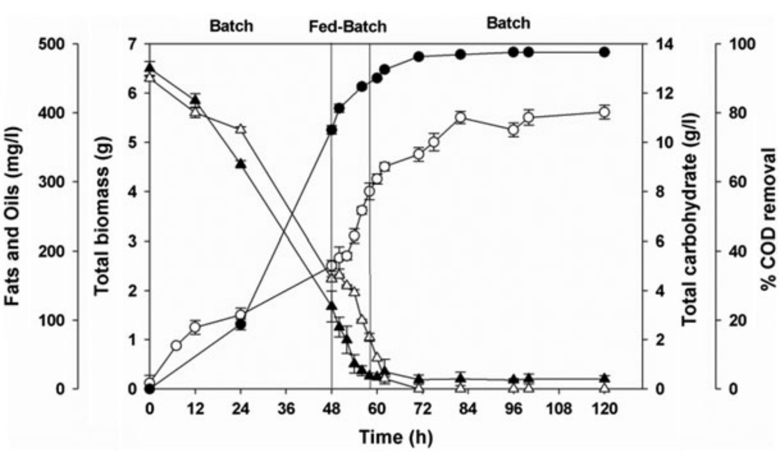

Fig. 3. Results of dairy wastewater treatment using the yeast-bioreactor system operated under fed-batch mode (▬○▬ Yeast biomass, ▬▲▬ Total carbohydrate, ▬△▬ Fat, ▬●▬ % COD removal).

wastewater was fed into the reactor at a rate calculated as per an earlier (Eq. (3)). A sharp increase in the yeast biomass was observed, which quickly utilized the fats and oils, and carbohydrate from the wastewater, which resulted in more than 95% COD removal at 60 h. In the third stage (60–120 h), the reactor was operated under batch mode, during which time fats and oils were completely removed from the wastewater.

Similar results have been observed in our earlier study [15], in which fed-batch operation of the reactor resulted in a quick and more efficient treatment of a synthetic dairy wastewater than when the reactor was operated under batch mode. This can be better explained based on the yeast biomass growth which was much higher than that in the batch experiments for the same time period (Fig. 3). Under the batch-operated condition, the yeast biomass in the reactor reached its stationary growth phase after 60 h and when the initially added carbon source was depleted (Fig. 2). In the fed-batch-operated reactor, due to the fresh supply of wastewater, the yeast continued to grow by utilizing the carbon source present in the wastewater. Hence, the results of biomass growth, removal of fats and oils, and COD from the wastewater were superior in the fed-batch-operated reactor than those obtained under batch-operated condition.

3.4. Removal of fats and oils under continuous operation

The results of fats and oils removal from the dairy wastewater under continuous mode of operation by the yeast *C. bombicola* are shown in Fig. 4. It could be seen that the continuous feeding was started after 72 h of batch process. At the end of batch process (72 h in Fig. 4), the residual concentration of total carbohydrate and fats and oils were less than 1 g/L in the wastewater. The yeast biomass concentration was more than 2.5 g/L. Although during the initial feeding of wastewater the yeast biomass reduced slightly, it then remained constant throughout the experiment due to which the yeast was able to utilize all the fats and oils present in the wastewater during this continuous feeding. Also, the concentration of sophorolipids produced and the COD removal efficiency were 1–2 g/L and >95%, respectively, which are similar to those observed in the batch and fed-batch experiments. Moreover, the COD removal efficiency (>95%) obtained in this study (using real wastewater) is even better than that reported in our earlier study using synthetic wastewater [15].

An added advantage of the present yeast-bioreactor system is that it can be used to treat such wastewater even under non-sterile conditions as all the experiments (batch, fed-batch and continuous) were conducted in a similar manner in this study, which suggests its practical application in wastewater treatment plants. However, a detailed cost-benefit analysis of this system will still be necessary in order to further establish its application potential.

Matsui et al. [25] studied the effect of different surfactants, including the biosurfactant saponin, on the continuous treatment of salad oil-containing synthetic wastewater by using activated sludge biomass. The authors found that the outlet oil concentration in the wastewater remained very low at 30 mg/L in the presence of surfactants compared to more than 100 mg/L without any added surfactant. Similarly, Nakhla et al. [8] studied the effect of commercial biosurfactant (BOD-Balance™) on the treatment of pet food wastewater by anaerobic digestion system and found that the biosurfactant at a dose in the range

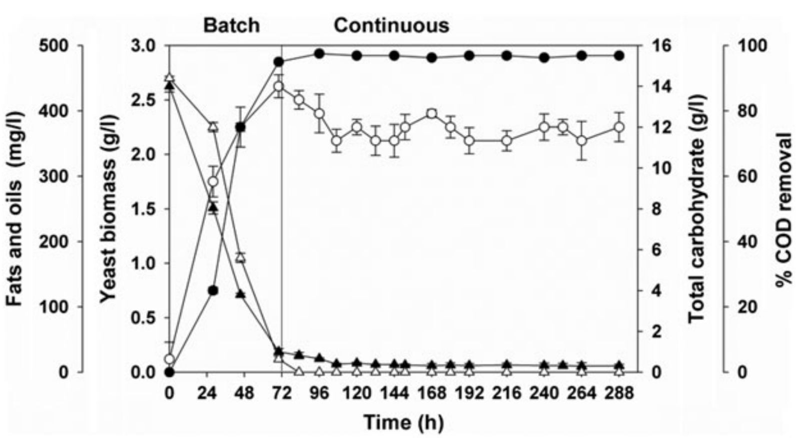

Fig. 4. Results of dairy wastewater treatment using the yeast-bioreactor system operated under continuous mode (▬○▬ Yeast biomass, ▬▲▬ Total carbohydrate, ▬△▬ Fat, ▬●▬ % COD removal).

130–200 mg/L decreased oil and grease concentrations from 66,300 to 10,200 mg/L along with a reduction in the initial COD up to 40.86% over a two-month operation period. Recently, Banu et al. [26] studied pretreatment of dairy wastewater by anaerobic hybrid up flow anaerobic sludge blanket reactor followed by its final treatment using solar photocatalytic method. The anaerobic pretreatment method was able to remove 84% of COD with the secondary solar photocatalytic treatment removing the remaining COD up to 95%. However, all these methods were reported to suffer from one or more drawbacks such as requirement of ex-situ addition of biosurfactant/coagulant/adsorbent, low COD removal efficiency, long pretreatment time, high operating cost etc.

4. Conclusions

Complete removal of fats and oils present in the dairy wastewater was achieved by the biosurfactant-producing yeast *C. bombicola* employing bioreactor operator under batch, fed-batch, and continuous mode of operations. Also, the yeast was able to remove COD of the wastewater with very high removal efficiency. Hence, this study established the very good potential of the biosurfactant-producing yeast *C. bombicola* in treating such high fats and oils containing wastewater without the need for an additional pretreatment step to remove the fats and oils.

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