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BIOREMEDIATION OF DAIRY EFFLUENT USING *CANDIDA INTERMEDIA* MTCC 1744 AND *KLUYVEROMYCES MARXIANUS* MTCC 3772

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ABSTRACT

The food sector is one of the highest consumer of water and also the biggest producer of effluent and sludge per unit production. Today, the annual production of processed milk in India is more than 150 million tons. The water requirement for washing and cleaning purpose is in the range of 0.9 to 2 liters of milk being processed (Kumar *et al.*, 2007). It has high organic load reflected in effluent levels of Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD_5), Oil and Grease, Nitrogen, Phosphorus (Noorjahan *et al.*, 2007). Physical treatment processes are employed, but biological treatment is cost effective, safe and it relies on microbes that naturally occur in environment (Adhya, 2008). Two yeast strains *Candida intermedia* MTCC 1744 & *Kluyveromyces marxianus* MTCC 3772 were employed for the present study and environmental conditions for maximum bioremediation were optimized. All the procedures were carried out as per standard AOAC (2005) methods.

INTRODUCTION

Industrialization is an important tool for the development of any nation. With the rapid growth of industry in the country, pollution of natural water by industrial waste has been increasing tremendously (Muthusamy *et al.,* 2001). Effluent generated causes water and soil pollution. The dairy waste generally contains large quantities of constituents such as casein, lactose, fat, inorganic salts besides detergent and sanitizers, which contribute largely toward high COD and BOD_5 (Noorjahan *et al.,* 2007). By applying various treatment technologies, the harmful effects can be reduced. BOD and COD should be reduced as per as BIS permissible limits by discharging waste water to inland surface waters, public sewers and for land irrigation.

Bioremediation is any process that uses living micro-organisms, or their enzymes to return a polluted environment to its original condition by breaking down organic matter. These microbes are helpful and pose no threat to people at the site or in the community. Yeast mainly *Candida, Saccharomyces, Kluyveromyces* sp. are known as effluent bioremediating agents.

Keeping the above facts in view, the present study was carried out with the following specific objectives of biologically treating the effluent using *Candida*

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intermedia MTCC 1744 and *Kluyveromyces marxianus* MTCC 3772 and also observing the environmental conditions under which they work best.

MATERIALS AND METHODS

The dairy effluent required for the experimental purpose was collected from Haryana Milk Food, Pehowa (Haryana) and Verka Milk Plant, Patiala (Punjab). It was filtered through ordinary filter paper to remove coarse solids. Effluent was stored at refrigeration temperature and not used beyond 72-96h. A metabolically active culture @ 1% (v/v) was taken for all trails and all the trails were done in duplicates. BOD_5 and COD were considered as parameters for judging the efficacy of the organisms.

Physical parameters as color, odor, turbidity, temperature, pH of the dairy effluent were observed at the time of collection. Chemical parameters observed include TDS, TSS, Oil & Grease, Biochemical Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD), Nitrogen, Phosphors and Total Carbohydrates. All the procedures were carried out as per standard AOAC (2005) methods. Microbiological analysis of effluent was done by using serial dilution techniques.

Candida intermedia MTCC 1744 and *Kluyveromyces marxianus* MTCC 3772 was procured from IMTECH, Chandigarh and grown on Malt Yeast Agar medium and incubated for 48h at 25°C. Dairy effluent is rich in microflora. Hence bioremediation by indigenous microflora alone and when supplemented with given yeast cultures were compared.

The efficacy of biological treatment would depend upon inoculum size and also environmental conditions primarily pH and temperature. There is needed to optimize cell biomass to be used in order to minimize cost incurred on the process. The effect of inoculum size; @ 1 %-7 % (v/v) on biological treatment of dairy effluent was studied.

The state of Punjab encounters all climate conditions in a year. Inoculated effluent was incubated at temperatures ranging from 10°C-45°C and BOD_5 and COD reduction was recorded.

Effluent exhibits pH over a wide range i.e. 5.2 to 9.0. It necessitates that the microorganism be able to grow in this wide range. So the pH from 4.0 to 9.0 was taken into account.

Inoculum size, temperature and pH of the effluent optimized earlier, were employed in this trial and minimum time period required to achieve appreciable BOD_5 and COD reduction was ascertained. Minimum retention time makes ETP feasible to run.

RESULTS AND DISCUSSION

The physicochemical and microfloral characteristics of the dairy industrial effluent vary with the season and also depend upon the type of dairy product being produced, nature of process, efficiency of processing procedure and quantity of milk being processed.

Effluent characteristics

Table 1 show the results of physico-chemical and micro-floral analysis of samples. The color of samples was dirty white to grey having unpleasant, pungent smell. The pH was ranged from 5.2 to 9.0. The pH is attributed to applications of detergents (caustic and washing soda extensively used in the dairy industry for washing purposes etc.) in varying concentration (Noorjahan *et al*., 2004). Dairy effluent having pH between 1.0-13.0 has also been reported (Briao and Granhen, 2007). The temperature of samples ranged between 16°C-36°C. It depends upon seasonal variations. The time period of effluent collection was March-July, 2008. Total Solids (TS) were ranged between 0.037-2.9 mg/mL, Total Suspended Solids (TSS) between 0.03-1.4 mg/mL, Total Dissolved Solids (TDS) between 0.007-1.5 mg/mL. The BOD5 of the effluents was observed to be ranged between 1057-2550 mg/L and COD 2000 mg/L- 4800 mg/L which is beyond government permissible limits. COD is 1.5 times the BOD_5 reading. Oil & Grease content was recorded to be 0.011-4.56 mg/mL. Nitrogen and Phosphorous content was measured between 0.017-0.030 mg/mL and 0.0104-0.783 mg/mL respectively.

These observations of the analysis of effluents are in concurrence with the results reported by various workers of dairy industry in Punjab, India (Harper *et al*., 1971; Kearney, 1973; Sethi *et al*., 1981 and Tiwana, 1985).

The microbial content of effluent depends on the management of hygiene in the plant. Maximum count of 1×10^6- 4×10^8 cfu/mL of bacteria, 3.1×10^6 cfu/mL of yeast and upto 5.1×10^5 cfu/mL of fungi was detected. Since the pH ranges obtained during this period varied between neutral to basic, they supported more growth of bacteria in comparison to yeast and mold. The source of microorganisms in effluent is raw milk itself, contaminants added in the production line by the equipment and personnel. The microbial count obtained can be due to unsanitary quality of water used during processing (Kumar, 1998).

Biological Treatment Technologies

To begin with effort was made to ascertain whether the chosen organism alone or in conjugation with the existing microflora can be more effective. Effluents collected from Haryana Milk Food, Pehowa containing indigenous microflora at work gave a BOD_5 reading 1057 mg/L and COD reading of 2000 mg/L. The BOD_5 reading of 236 mg/L and COD of 737 mg/L with the addition of Candida intermedia MTCC 1744 (BOD_5 reduction of 77.67% and COD of 63.15%) was achieved (Figure 1(a)). It was much more than that achieved by this organism alone, i.e. BOD_5 reduction of 37.5% and 45.25% COD reduction.

Effluent collected from Verka Milk Plant, Patiala containing indigenous microflora with *Kluyveromyces marxianus* MTCC 3772 gave BOD_5 of 657 mg/L and COD was 1500 mg/L compared to *Kluyveromyces marxianus* alone in sterilized effluent, i.e. BOD_5 of 853 and 1920 mg/L (an improvement of 74.20% BOD_5 and 68.70% COD with use of *Kluyveromyces marxianus*) (Figure 1(b)). The effluent appeared as clear liquid after this biological treatment as shown in (Figure 2).

Many investigations have been done on *Candida* sp. and similar observations have been made by workers with different yeast cultures as well as Duck in 2003. *Candida* sp. was used as mixed cultures for treatment of whey; its batch treatment produced a high yield of biomass and a greater removal of BOD_5 and COD removal efficiency i.e. 95.8% (Marwaha *et al.*, 2001).

Optimization of process parameters for the treatment of dairy effluent Inoculums size

With *Candida intermedia*, maximum BOD_5 and COD reduction was achieved with 5 % (v/v) inoculum rate i.e. 77.0% and 81.78% corresponding to 7 x 105cfu/mL (Fig 3(a)).

In case of *Kluyveromyces marxianus*, maximum BOD_5 and COD reduction was exhibited by 6.0 % (v/v) inoculum rate i.e. 86.20 % and 80.00 % respectively correspond to approx. 8.5 ×105 cfu/mL (Figure 3(b)). Using more than required level of inoculum is uneconomical and also the cell biomass may clog the pipes in the drainage system in ETP causing secondary pollution problems.

Temperature

Different temperatures were employed to observe the environmental effect on BOD_5 reduction of dairy samples i.e.10°C to 45°C inoculated @ 5% (v/v) Candida intermedia and 6% (v/v) Kluyveromyces marxianus as optimized.

Candida intermedia gave maximum BOD_5 reduction at temperature 25°C i.e. 47.90% and minimum reduction was obtained at temperature 37°C i.e. 5.3%. At 30°C, BOD_5 reduction was 33.7%. The temperature 25°C had been earlier demonstrated by this yeast as its optimum temperature of growth (Figure 4). Maximum reduction with Candida intermedia was achieved at 25°C. Below and above this temperature, yeast has a slower growth rate which reduces its bioremediation potential.

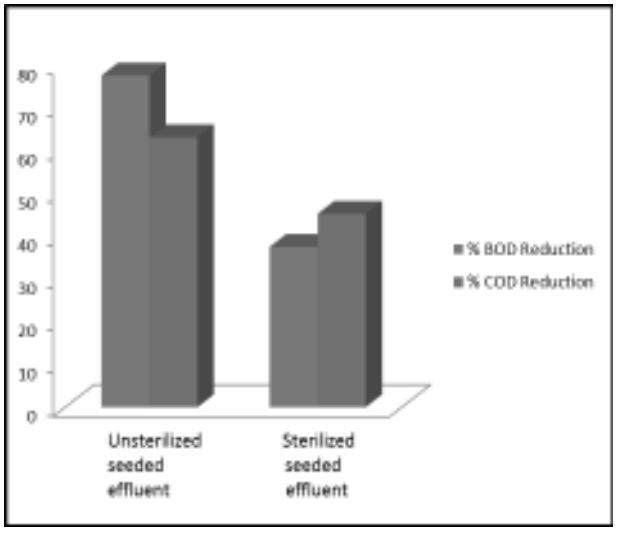


Fig. 1(a) Comparative treatment of dairy effluent using *Candida intermedia* MTCC 1744

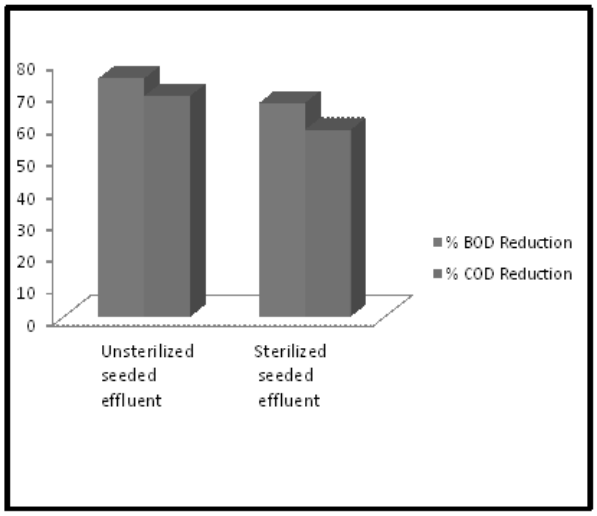


Fig. 1(b) Comparative treatment of dairy effluent using *Kluyveromyces marxianus* MTCC 3772

Table 1. The Physical, Chemical and Micro-floral characteristics of Dairy Effluent.

S.No.	Characteristics	Observation
1.	Color	Dirty white to grey
2.	Odor	Pungent
3.	pH	5.2-9.4
4.	Temperature	16°C-36°C
5.	Total solids (TS)	0.037 -2.9 mg/mL
6.	Total Suspended Solids	0.03-1.4 mg/mL
7.	Total Dissolved Solids	0.0072-1.5 mg/mL
8.	Oil and Grease	0.011-4.56mg/mL
9.	Nitrogen	0.03024 -0.17mg/mL
10.	Phosphorous	0.01-0.783 mg/mL
11.	BOD_5	1057-2550 mg/L
12.	COD	2000-4800 mg/mL
13.	Bacteria	1 ×106- 4 x 108 cfu/mL
14.	Fungus	Nil to 5.1 x 10^5 cfu/mL
15.	Yeast	Nil to 3.1 x 10^6 cfu/mL

Sample A-Untreated Effluent Sample B-Treated Effluent

Fig. 2 Biologically treated dairy effluent using *Candida intermedia* MTCC 1744 and *Kluyveromyces marxianus* MTCC 3772

The efficiency of yeast Kluyveromyces marxianus to remediate the dairy effluent at temperature 25°C and 30°C is maximum i.e. 68.6 % and 74.4 % respectively. Below 25°C and above 35°C, it has lower growth rate which does not boost BOD_5 and COD reduction (Figure 4).

The similar temperatures have earlier been reported optimum for the treatment of dairy effluent using *S. fragilis* (Sethi *et al.*, 1981). The temperature 25°C-30°C has been reported optimum for treatment of dairy effluent using *Candida parasilopsis* MTCC 1965 (Marwaha *et al.*, 2001). BOD_3 (72.09%) and COD

Fig. 3 (a) Reduction in BOD_5 and COD with inoculum size as a function using *Candida intermedia* MTCC 1744

Fig. 3(b) Reduction in BOD_5 and COD with inoculum size as a function using *Kluyveromyces marxianus* MTCC 3772

Fig. 4 Reduction in BOD_5 with temperature

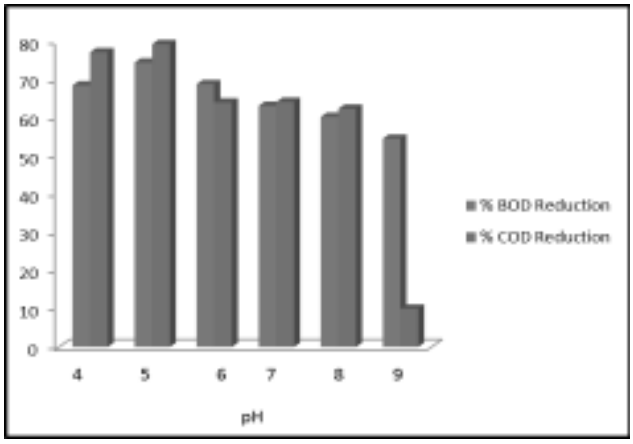
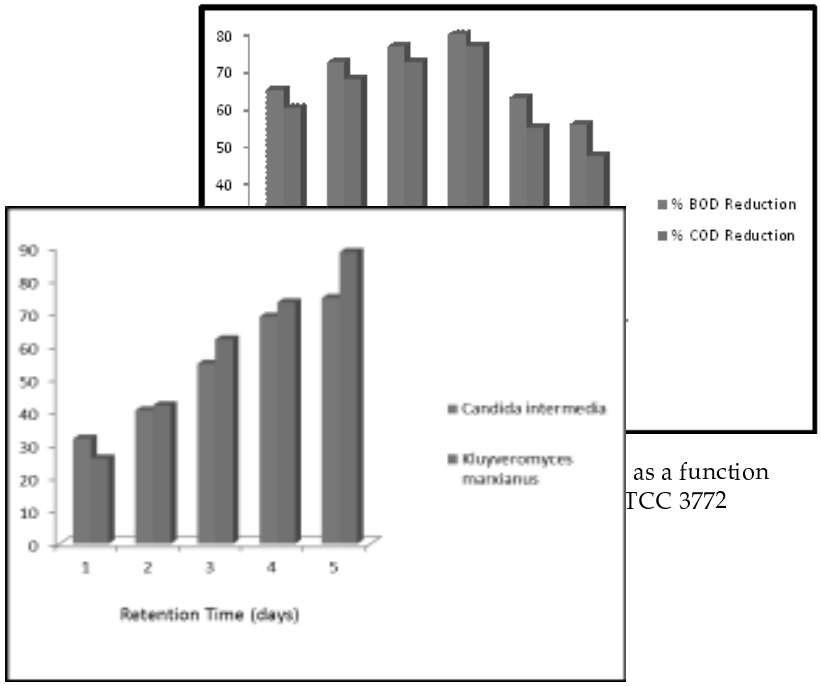


Fig. 5(a) Reduction in BOD_5 with pH as a function using *Candida intermedia* MTCC 1744



as a function
TCC 3772

Fig. 6 Retention Time and Reduction in BOD_5

(70.62%) removal of dairy waste using free cells of *C. parasilopsis* at 30°C and pH 5.5 with inoculum size 10% after 24 hrs (Ghosal *et al.*, 1995).

pH

Candida intermedia showed greatest BOD_5 and COD reduction with pH 5.0 i.e. 74.45% and 79.25% respectively. pH 5.0 and 6.0 are optimal for growth of native microflora and hence it along with our organism works best at these pH. At markedly alkaline pH of 9.0, the reduction is down to 54.5% BOD_5 and 10% COD in this study (Figure 5(a)).

Kluyveromyces marxianus gave comparable results at pH 5.0, 6.0, 7.0 i.e. 71.60%, 75.90%, 79.20% respectively and COD reduction was 67.30%, 71.60%, 76.0% respectively. (Figure 5 (b)).

The pH 4.5 has been shown to be optimal for the treatment of dairy effluents in studies of Sethi *et al.*, (1981) using *S. fragilis*. Lower degree of bioremediation at higher pH can be attributed to unfavorable physiological environment and also their effect on RNA and protein biosynthesis (Klovrychev *et al.*, 1979).

Application of all the optimized parameters

Effluent was biologically treated with the above optimized parameters so as to arrive at minimum retention time required to give maximum BOD_5 reduction was ascertained. On fourth and fifth day, *Candida intermedia* gave BOD_5 reduction 68.77% and 74.4% respectively. *Kluyveromyces marxianus* gave maximum BOD_5 reduction after fifth day incubation that is 88.30 % (Figure 6).

Maximum reduction of BOD_5 (74.6%) of dairy effluents in 72h using *S. fragilis* has been reported (Vananual *et al.*, 1975). The time 20h also has been reported with *Candida parapsilosis* MTCC 1965 under shake flask conditions (Marwaha *et al.*, 2001). *Alcaligens* sp. gave maximum reduction in COD (62%) in 5th day of incubation (Rajeshkumar *et al.*, 2003).

Hence minimum retention time is desirable for quicker disposal and economy of running the ETP. Further work needs to be further minimizing retention and also to immobilize the microflora on a matrix to come up with a technology for a continuous system.

CONCLUSION

Milk production in India is 108.5 MT per annum with close to a share of 20% of the total world production

in the year 2008-09 (NDDB, 2009). It supplements the income of farmers but also generates waste containing large number of impurities, which contributed towards high pollution potential. The parameters used to measure the pollution potential are Biological Oxygen Demand (BOD_5) and Chemical Oxygen Demand (COD). The effluent samples collected over the period of study exhibited varied physical, chemical and microfloral characteristics. *Candida intermedia* produce maximum bioremediation benefits at 37°C temperature, 5.0 pH and with inoculum level of 5.0% (v/v) and in case of *Kluyveromyces marxianus*, best treatment of effluent was achieved at 30°C temperature, 7.0 pH with inoculum level 6.0% (v/v). A retention time of 4 -5 days is required to get appreciable bioremediating benefit. Further work on immobilization of the biomass and development of a continuous system needs to be done.

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