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RESEARCH ARTICLE

In situ Bioremediation of Dairy Waste Water–A Novel Approach in Dairy Waste Management

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Abstract

A steady rise in the demand for milk and milk products in many countries has led to advancements in veterinary science and these demands are sufficed by the growing dairy industries. Dairy industry is one of the major industries causing water pollution. Considering the increased milk demand by 2020 A.D., the milk based food industries in India are expected to grow rapidly so the waste generation and related environmental problems associated with this will also gain increased importance. Poorly treated wastewater with high levels of pollutants caused by poor design, operation or treatment systems creates major environmental problems when discharged to surface water or land. Considering the above stated implications an attempt has been made in the present study to mitigate the major problem of elevated COD level in dairy wastes. Raw Effluent [RE] samples were collected from the Saras Dairy (Jaipur) plant and parameters like pH, temperature, alkalinity, acidity, chloride, hardness and COD were analyzed for evaluation of pollution level. Research was conducted to study the indigenous waste water microbiota and to identify some active strains adapted to the physical and chemical conditions of the same for using them as specialized inoculums in wastewater treatment on test models, in laboratory bioreactors or pilot plants. The biodegradation assay revealed the potential of the isolated cultures to metabolize organic compounds, similar to those present in food industry wastewaters. Strains able to produce a fast biodegradation of the organic compounds were isolated and identified.

Keywords : Biological treatment, *Citrobacter sp*, COD, Dairy WWTP, *in situ* bioremediation, Raw effluent

Introduction

With advances in veterinary sciences, production of milk has tremendously increased to cope up with the continuous increasing demand of consumers. But the growing dairy industry increased the burden on the environment in the form of generated waste products. The resultant toxic substances, generally released in the form of solids, liquid effluent and slurries containing a spectrum of organic and inorganic chemicals are hazardous to health and also affect the growth of flora and fauna. Industrialization of nations coupled with rapid acceleration in population growth, has led to severe problems of environmental degradation including water pollution. Like any other food industry, dairy industry faces the problem of having relatively less number of waste treatment plants. Liquid effluents from these industries immensely pollute water and soil, and pose serious environmental problems.

Dairy effluents have shown presence of high organic load that is responsible for rapid depletion of the dissolved oxygen (DO) level of the receiving streams. Not only this, they also become the propagation site for mosquitoes and flies causing malaria and other perilous diseases like dengue fever, yellow fever and chikungunya (Kumar and Desai, 2011; Bhadouria and Sai, 2011). Several sub plants of the dairy industry such as the receiving station, bottling plant, cheese plant, butter and ice cream plant, generate contaminating water pollutants such as fat, milk proteins, lactose, lactic acid, minerals, detergents and sanitizers (Arceivala, 1980; Banu *et al*, 2008). Nutrients like nitrogen, present in dairy effluents lead to eutrophication of the receiving water bodies and detergents and sanitizers affect the aquatic life (Kushwaha *et al*, 2011). Not only this, nitrogen from dairy wastes can also contaminate ground water with nitrate as it can be converted to the latter (Ulery *et al*, 2004).Nitrogen is harmful for human health in any of the following forms ammonia, nitrite or nitrate, and raw milk contains about 3–8mg/L of nitrogen in the form of ammonia. With only an estimated 1% loss of milk, about 50 mg/L of nitrogen can be present in wastewater stream (Wendorff, 1998). Nitrite is also known to cause intestinal cancer and nitrate can cause methanoglobinemia if converted to nitrite (Breisha, 2010).

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The degree of pollution and the sanitary requirements are important factors to decide if wastes can be either discharged straight into a stream or they require an appropriate treatment that can be mechanical, chemical, or biological. The presence of a relatively high fraction of readily biodegradable organic compounds in the dairy plant wastes, responds positively to the biological treatment. Several biological methods have been thoroughly studied to provide efficient treatment to wastes. These methods include treatment with an up-flow anaerobic sludge blanket reactor (Mehrotra and Jain, 1997), denitrification using microbial fauna (Zayed and Winter, 1998), continuous aerobic treatment (Carta-Escobar *et al*, 1999), thermophilic acidification using an up flow reactor (Fang,1990) and treatment with water hyacinth (Trivedy and Pattanshetty, 2002) and many more.

Due to depletion of DO, oxidation of organic matter in the dairy wastewater is hampered and in such cases lactose when converted to lactic acid, lowers the pH of the medium to an extent that casein is precipitated. The resultant anaerobic decomposition of protein yields foul-smelling substances in the water, which neither support aquatic life nor render it suitable for drinking purpose. Therefore, it becomes a necessity to efficiently treat dairy wastewater in order to reduce its organic load before being discharged into nearby waterways.

With the concomitant increase in demand of milk and related products, dairy ventures in India have grown rapidly to provide pasteurized milk and produce a wide range of processed milk products. These processing methods in turn generate organic wastes and physico-chemical analysis show high levels of COD in the treated waste. The small biological methods based treatment plants seems to be the most adequate solution to remediate this environmental issue.

Biological treatment is an economical alternative to physical and chemical methods for treatment of dairy wastewater. It is essential to remove organic matter from dairy effluents before allowing its waste water to enter other close proximal water bodies. The degradation of colloidal and dissolved substrates by the available microorganisms is the underlying mechanism of any biological treatment. The susceptibility of organic substances present in the dairy wastewaters, to biochemical oxidation by microorganisms, is a precondition for proficient bioremediation. Use of single microorganism may not fulfill the purpose of biodegradation. There may be requirement for specific and mixture of microorganisms that produce cumulative effect in biodegradation of the organic contaminants present in dairy wastes. This can be due to the combined effect of accelerated biomass activity, growth efficiency and microbial enzyme production. Treating waste using biological methods is the most widely used process not only for removal but also for partial or complete stabilization of biologically degradable substances present.

In the present investigation, we tried to develop a biotechnological approach to treat the dairy wastewater using a microbial milieu that possesses efficient biodegradation potential for biological treatment of dairy wastewater. The effort was to minimize COD values to the recommended environmental standards by employing surface active materials like alum in conjugation with microbial treatment.

Materials and Method

Collection of dairy waste water samples

Effluent samples of dairy wastewaters were collected from Saras dairy plant, located in Jaipur, Rajasthan, India. It is one of the largest dairy ventures in India. Collected effluents were processed as per Grab Methodology [APHA] (Lenore *et al*, 1998). The samples were collected in sterilized containers from the settling tank in the early hours of the day, as soon as the wastewater from the milk-processing unit was pumped into the tank.

Physicochemical studies

Physico-chemical studies of the wastewater were carried out according to the standards of American Society for testing wastewater samples [APHA] (Lenore *et al*, 1998). Parameters like most probable number (MPN), pH, temperature, alkalinity, acidity, chloride content, hardness and COD were analyzed.

Isolation of various bacterial strains

Bacterial strains were isolated from the wastewater by using serial dilution method. Effluent was centrifuged at 6000 rpm to remove the coarse material and supernatant was used for plating on nutrient agar media (Himedia), according to the serial dilution method. The cultured plates were incubated at 37°C for 24-48 hrs. The colonies with distinct morphology were observed and re-streaked (quadrant) on nutrient agar plates in order to obtain pure cultures. The bacteria thus isolated were then subjected to various biochemical identification parameters. Biochemical tests such as indole, citrate utilization, lactose and glucose fermentation tests were done to study unique biochemical characteristics of bacterial strains. The isolated cultures were grouped into various genera on the basis of their morphological and biochemical characteristics as outlined in Bergey's Manual (Holt *et al*, 2000).

Treatment of waste water

Pretreatment of the effluent- In our study we used aluminium potassium sulfate, also known as alum (Sigma) a mineral salt adjuvant-coagulant for the primary treatment of wastewater to reduce the amount of organic chemical load of the latter. The effluent water was treated with alum at a concentration of 0.25-1.25 gm/100 ml and reduction in COD values was calculated.

Biological treatment- Treatment with free bacterial cells and selection of efficient isolate:

The bacterial strains so far isolated were used to biologically treat dairy effluents. In order to determine the efficiency of bacteria to reduce the levels of COD, bacterial cell suspension of known concentration was used in inoculated with alum treated effluent. 800 ml of the effluent was inoculated with 1% (v/v) primary inoculum of cell concentration (1×10^7) and incubated at 28°C) for 5 days. A small volume of samples was withdrawn at regular intervals of 24 h and analyzed for COD value. The efficiency of biological treatment was expressed in terms of reduction in COD value. The isolate that gave maximum reduction in COD values of wastewater during similar conditions was selected as the most potential strain, capable of providing efficient bioremediation.

Identification of selected bacterial strain

The isolated bacteria that showed maximum reduction in COD was identified by 16S rDNA analysis. For 16S rDNA sequencing, the bacterial culture was inoculated in Luria Bertani broth (Himedia) for 12 hr and used for isolating total DNA isolation using Genomic DNA Extraction kit (Fermentas). Universal primers were used to amplify 16S rDNA gene (Table 1). Following conditions were used for carrying out polymerase chain reaction, denaturation-94°C for 1 min, annealing-55°C for 1 min, and extension-72°C for 2 min, with 40 repeated cycles. The amplified gene was gel purified using gel extraction kit (Qiagen) and sequenced. The sequence data was analyzed by BLAST at NCBI. The bacterium was then accordingly identified based on similarity with the sequenced database.

Results and Discussion

Physical and chemical analysis- The physicochemical analysis of the dairy waste water samples was as presented in table 2. The COD indicated the quantity of the pollutants in waste water that could be oxidized by a chemical oxidant moiety. The results clearly indicated the need to treat dairy wastewater before discharging into water ways. The high organic load was mainly due to dissolved milk protein and sugars and therefore, there was a great necessity to treat the effluents before being discharged. The biological methods displayed application for dairy waste water treatment and its efficient bioremediation.

Table 1. PCR carried out with paired primers

S.No	Forward	Backward	Identity
DRS1	TCTGAGAGGA TGACCAGCCA	GGGACTTAACC CAACATTTC	*Citrobacter*
DRS2	AGAGTTTGATC CTGGCTCAG	CTTGTGCGGGCC CCCGTCAATTC	*Escherichia*
DRS3	TACCTACAAC CTCAAGCT	TACCCATTCTAA CCAAGC	*Proteus*

Isolation of Bacteria - Dairy effluents were screened and three strains were isolated. They were named as DRS1, DRS 2 and DRS 3 according to the site and order of isolation. All of them were gram negative rod-shaped bacteria. These were identified on the basis of morphological and biochemical analysis, up to the genus level as *Citrobacter* (DRS 1), *Escherichia* (DRS 2) and *Proteus* (DRS 3).

Table 2. Physico-chemical parameters

S.No	Parameters Studied	Raw Sample	Permissible limit(WHO)
1.	MPN (per 100ml)	<5	-
2.	pH	7.5	6.5-8.5
3.	Temperature	30±0.5° C	-
4.	Alkalinity	123.66±3.24 mg/l	200
5.	Acidity	10.2 ±0.20 mg/l	-
6.	TDS	132 mg/l	200 mg/l
7.	Chloride	333.7±2.34 mg/l	200 mg/l
8.	Hardness	232 ±4.35 mg/l of $CaCO_3$	300 mg/l
9.	COD	217.5±2.78 mg/l	150 mg/l

Pretreatment by using alum- The mechanism behind coagulation treatment is that a chemical substance when added to an organic colloidal suspension causes its destabilization by the reduction forces that kept them apart. It involved the reduction of surface charges, liable for particle repulsion and causes agglomeration. Particles of larger size settle and clarify effluent received (Zayed and Winter, 1998).We used alum at varying concentrations and found that at 0.5 g/100 ml concentration, it was most effective in reducing COD level of the collected dairy effluent. The result of coagulation was visible in the form of porous gelatin flake deposits that settled at the bottom of the flask and reduce the levels of COD of the effluent (Fig.1).

Biological treatment- Once pre-treatment with alum was done, inoculation with three different isolates viz. DRS1, DRS2 and DRS3 of the effluent water samples was carried out and COD values were recorded accordingly. This was done to determine the efficient bacterial isolate that gave

maximum reduction in the levels of COD in the stipulated 5 days (Fig.2).

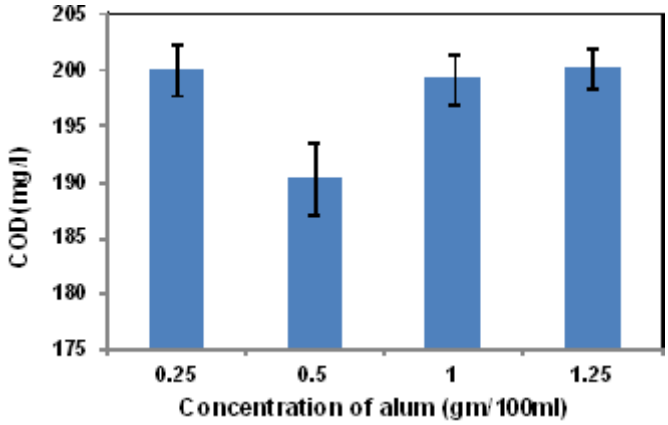

Fig.1. COD values of the dairy effluent samples after treatment with different concentrations of Alum

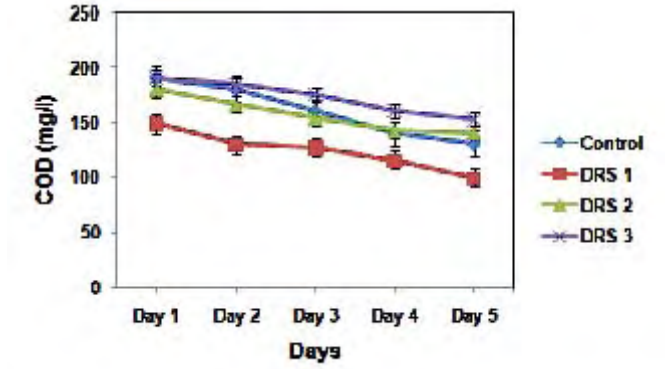

Fig.2. COD values of the control and treated samples with 1% inoculation with different isolates.
DRS1- Dairy Raw Sample 1(10^{-3}), DRS2- Dairy Raw Sample 2(10^{-3}), DRS3- Dairy Raw Sample 3 (10^{-3}). *P<0.05, ** P<0.001, ns=non significant

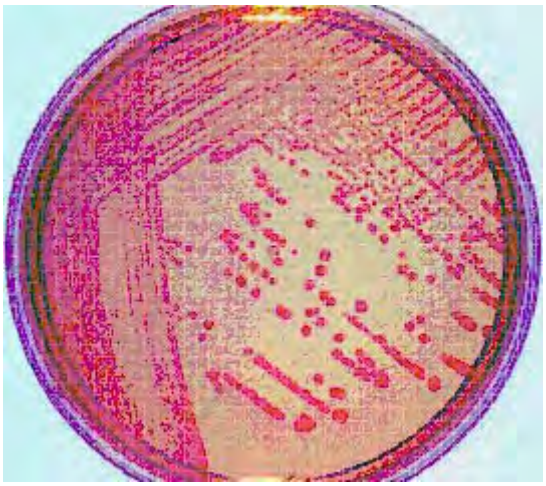

Fig.3. DRS1 (*Citrobacter*) morphology when plated on MacConkey Agar Plate

Out of the three, the bacterial isolate designated as DRS1 and later identified on the basis of 16S rDNA gene sequence, as *Citrobacter* sp. was found to be the most potent isolate (Fig.3). The COD value of the control sample (with no microbial inoculation) was 190.5± 2.78 mg/l at Day 0. On treatment with DRS1 isolate, COD values obtained were 148.8 mg/l (Day 1), 130 mg/l (Day 2), 127.2mg/l (Day 3), 115.9mg/l (Day 4), and 99.7 mg/l (Day 5)(Fig.2). The COD value thus showed a continuous declining pattern with increased time of incubation when treated with DRS1 culture.

The isolate DRS1 i.e. *Citrobacter* sp., was most efficient in the consortium of isolated bacterial strains and significantly reduced COD value as compared to control.

Conclusion

Dairy effluents have high organic load content and unscrupulous discharge of this waste water into other water bodies is a matter of great concern as this deteriorates water quality and is hazardous to aquatic life as well as for human and animal consumption. In an attempt to remove the suspended solids from the effluent, we employed chemical coagulation using alum as a primary treatment method. This was followed with biological treatment in which microorganisms isolated from the wastewater were used for treating the dairy effluents. This is often very helpful as the selected organisms are already acclimatized to the milieu and thus exhibited better efficiency in bioremediation of wastewaters. This microbial consortium was capable of effectively reducing the pollutant load of dairy wastewaters, as indicated in terms of COD values.

The best selected bacterial species *Citrobacter* sp., showed maximum reduction of COD levels and can therefore be developed as an appropriate biotechnological tool to be used for large-scale treatment of dairy wastewaters.

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