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# Treatment of Dairy Wastewater Using a Selected Bacterial Isolate, *Alcaligenes* sp. $MMRR_7$

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## Abstract

Physicochemical and biologic analysis of dairy wastewater showed that the effluent had a high organic load (chemical oxygen demand [COD]: 5095 mg/L), an acidic pH (6.4), and a high probability of coliforms (most probable number [MPN] > 1100). The various bacterial strains isolated and purified were identified as *Sporolactobacillus* sp., *Citrobacter* sp., *Pseudomonas* sp., *Alcaligenes* sp., *Bacillus* sp., *Staphylococcus* sp., and *Proteus* sp., as per the Bergey's manual of systematic bacteriology. Among the five selected bacterial strains, the strain designated as $MMRR_7$ and identified as *Alcaligenes* sp. was found to give a maximum reduction in COD (62%) in 5 d of incubation. Chemical coagulation using alum at a concentration of 0.5 g/100 mL was found to be effective in the primary treatment of the effluent. Studies on free-cell treatment of the coagulated effluent with the selected bacterial strain *Alcaligenes* sp. $MMRR_7$ gave a maximum COD reduction of 91% in 120 h. This study clearly indicates the possibility of using *Alcaligenes* sp. $MMRR_7$ for the effective treatment of dairy wastewater.

**Index Entries:** Dairy waste; wastewater treatment; biologic methods; free-cell treatment; *Alcaligenes* sp.

## Introduction

Water is one of the most important requirements of humans, and there is a dearth of pure water resources around the world owing to continuous pollution *(1)*. Dairy waste carries fat, milk proteins, lactose, lactic acid, minerals, detergents, and sanitizers *(2)*. The liquid waste of dairies originates from several sources such as the receiving station, bottling plant,

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cheese plant, butter plant, and ice cream plant *(3)*. When dissolved oxygen is insufficient for oxidation of organic matter in the dairy wastewater, lactose is converted into lactic acid, which, in turn, lowers the pH to a point where casein is precipitated. The resultant anaerobic decomposition of protein yields foul-smelling substances, which neither will support fish life, nor will animals drink this water. Hence, it is highly essential to reduce the organic load of dairy wastewater before being discharged into nearby waterways.

Most dairy plant wastes respond to the biologic treatment approach owing to the relatively high fraction of readily biodegradable organic compounds. Many biologic methods, such as treatment with an upflow anaerobic sludge blanket reactor *(4)*, denitrification *(5)*, continuous aerobic treatment *(6)*, thermophilic acidification using an upflow reactor *(7)*, and treatment with water hyacinth *(8)*, have been reported for the treatment of dairy wastewater. However, 50% of the dairy units have yet to attain satisfactory performance levels regarding installation and operation of effluent treatment plants *(3)*. Hence, it is relevant to explore new possibilities of treating dairy wastewater. The objectives of the present study were physicochemical and biologic analysis of dairy wastewater and free-cell treatment of the wastewater with a newly isolated and characterized bacterial strain.

## Materials and Methods

### *Sample*

Samples of a raw effluent from a milk-processing unit were generously supplied by the Manjoormilk Milk Plant, Manjoor, Kottayam, Kerala. The samples were collected in sterilized containers from the settling tank in the early hours of the day, when the wastewater from the milk-processing unit was pumped into it.

### *Estimation of Physicochemical Parameters*

Physicochemical studies of the wastewater were carried out according to the American Society for Testing and Materials methods *(9)*.

### *Bacteriologic Analysis*

#### Isolation and Identification

Effluent samples collected for bacteriologic analysis were used as inoculum after thoroughly shaking the containers for 15 min in order to ensure mixing of the sample. The bacteriologic studies were carried out by serial dilution followed by plating. The isolated cultures were grouped into various genera based on their morphologic and biochemical characteristics (Table 2) as outlined in the *Bergey's Manual of Determinative Bacteriology (10)*.

Selection of Potential Strains

Potential bacterial strains capable of growing and reducing the chemical oxygen demand (COD) rapidly in the raw effluent were considered as potential strains for future studies. Hence, five of the strains selected after primary screening from effluent samples were subjected to further screening by testing their ability to grow and reduce COD in the raw effluent in unsterilized conditions. One hundred milliliters of the effluent dispensed in a 250-mL conical flask was inoculated with a cell suspension of 1 optical density (OD) concentration ($1 \times 10^7$ cells) at a 1% (v/v) inoculum level and incubated at room temperature (28 ± 2°C) for 5 d. The strain that gave a maximum reduction in COD in the wastewater during the same set of conditions was selected as the potential strain.

### *Primary Treatment of Dairy Wastewater*

Several substances, such as aluminum sulfate, ferric chloride, or ferric sulfate, can be used as chemical coagulants for the primary treatment of wastewater *(11)*. In the present study, aluminum potassium sulfate (alum) was used as the coagulant. Dairy wastewater was treated by coagulation with aluminum sulfate at different concentrations, and the reduction in COD was calculated. The minimum amount of alum, which gave a maximum percentage of COD reduction, was selected as the optimum weight of the alum for the primary treatment of dairy effluent.

### *Treatment With Free Bacterial Cells*

The efficiency of bacteria to reduce levels of COD was determined by inoculation of unsterilized effluent with a cell suspension of known concentration. One hundred milliliters of the effluent dispensed in a 250-mL conical flask was inoculated with a cell suspension of 1 OD concentration ($1 \times 10^7$ cells) at a 1% (v/v) inoculum level and incubated at room temperature (28 ± 2°C) for 6 d. Samples were withdrawn at regular intervals of 24 h and analyzed for COD. The efficiency of treatment is expressed in terms of percentage reduction in COD. The growth pattern of the selected organism in the dairy wastewater was also studied and expressed as OD at different incubation times.

## Results and Discussion

The physicochemical and bacteriologic analysis of the dairy wastewater samples is presented in Table 1. The results clearly indicate the need to treat dairy wastewater before discharging into waterways. The high organic load is mainly owing to the dissolved milk protein and sugars. This also contributed to the proliferation of coliforms even at acidic pH. Because most of the organic load is owing to the dissolved proteins and sugars, there is a high scope for the application of biologic treatment methods for dairy wastewater.

Table 1
Physicochemical and Bacteriologic Analysis of Dairy Wastewater

| Serial no. | Parameter | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Average |
|---|---|---|---|---|---|---|
| 1. | THBP (CFU/mL) | $1.76 \times 10^4$ | $1.60 \times 10^4$ | $1.72 \times 10^4$ | $1.65 \times 10^4$ | $1.68 \times 10^4$ |
| 2 | MPN | >1100 | >1100 | >1100 | >1100 | >1100 |
| 3 | pH | 6.4 | 6.3 | 6.4 | 6.4 | 6.4 |
| 4 | Temperature (°C) | 27 | 26 | 25 | 25 | 26 |
| 5 | TSS (g/L) | 0.30 | 0.37 | 0.24 | 0.26 | 0.29 |
| 6 | COD (mg/L) | 7020.75 | 2538 | 3271.74 | 7548.77 | 5095 |

*Abbr:* MPN, most probable number; TSS, total suspended solids; COD, chemical oxygen demand.

Eleven strains randomly isolated during the bacteriologic analysis of the effluent were purified and subjected to identification. The various biochemical tests done were catalase, oxidase, indole, methyl red, Vogesproskeaur, citrate reduction, starch hydrolysis, oxidation/fermentation, $H_2S$, urease, triple sugar iron agar, and sugar fermentations based on *Bergey's Manual of Determinative Bacteriology* (Table 2). From the five strains obtained after primary screening, a potential strain was selected based on the efficiency to reduce COD to a maximum extent in 5 d (Fig. 1). The bacterial strain designated as $MMRR_7$ and identified as *Alcaligenes* sp. was thus selected as the potential strain.

According to Montgomery *(12)*, estimation of biologic oxygen demand is not ideally suited for studies on process design, treatability, control of treatment plants, setting of standards for treated effluents, and assessment of the effect of polluting discharges on the oxygen resources of receiving waters. Hence, in the present study COD was measured to determine the impact of the treatment system on the effluent.

In coagulation operation, a chemical substance is added to an organic colloidal suspension to cause its destabilization by the reduction of forces that keep them apart. It involves the reduction of surface charges responsible for particle repulsion and causes agglomeration. Particles of larger size are then settled and clarified effluent is obtained *(11)*.

In an attempt to remove the suspended solids from the effluent, we employed chemical coagulation as a primary treatment method. Alum was used as the coagulant, and Fig. 2 shows the effect of different concentrations of alum in bringing down the COD of dairy wastewater. Figure 2 shows that the alum at a concentration of 0.5 g/100 mL is more effective in reducing COD of the effluent. The visible result of coagulation is the formation of a deposit in the form of porous gelatin flakes that settle at the bottom of the vessel and thereby reduce the COD of the effluent.

Alum-treated dairy wastewater was employed for free-cell treatment using the identified and selected potential strain *Alcaligenes* sp. $MMRR_7$. During free-cell treatment, the COD was reduced to 40% in 24 h of incuba-

Table 2
Biochemical and Morphologic Features of Bacterial Isolates From Dairy Wastewater (based on *Bergey's Manual of Determinative Bacteriology*)

| | Motility | Shape | Gram staining | Catalase | Oxidase | Indole | MR | VP | Citrate utilization | Starch | O/F | $H_2S$ | Urease | Spore | TSIA | Lactose | Glucose | Sucrose | Results (genus) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $MMRR_1$ | + | Rod | + | − | + | − | + | − | − | + | F | − | − | + | + | − | − | − | *Sporolactobacillus* sp. |
| $MMRR_2$ | + | Rod | + | − | − | − | + | − | − | + | F | − | − | + | + | − | − | − | *Sporolactobacillus* sp. |
| $MMRR_3$ | + | Rod | + | − | − | − | + | − | − | + | F | − | − | + | − | − | − | − | *Sporolactobacillus* sp. |
| $MMRR_4$ | + | Rod | + | + | − | − | − | + | − | − | − | − | − | − | − | − | − | − | *Sporolactobacillus* sp. |
| $MMRR_5$ | + | Rod | − | − | + | + | + | − | + | + | F | − | − | − | + | + | + | + | *Citrobacter* sp. |
| $MMRR_6$ | + | Rod | − | + | + | − | − | + | + | + | O | − | − | + | − | − | − | − | *Pseudomonas* sp. |
| $MMRR_7$ | − | Rod | − | − | − | − | − | − | − | − | O | − | − | − | − | − | − | − | *Alcaligenes* sp. |
| $MMRR_8$ | + | Rod | + | + | − | − | − | + | + | + | F | − | − | + | + | − | − | − | *Bacillus* sp. |
| $MMRR_9$ | − | Spherical | + | + | − | − | − | + | − | − | F | − | + | − | + | − | − | − | *Staphylococcus* sp. |
| $MMRR_{11}$ | − | C.B. | − | − | − | − | − | + | + | − | − | − | − | − | − | − | − | − | *Alcaligenes* sp. |
| $MMRR_{12}$ | + | C.B. | − | + | − | − | − | + | + | − | O | + | + | − | + | − | − | − | *Proteus* sp. |

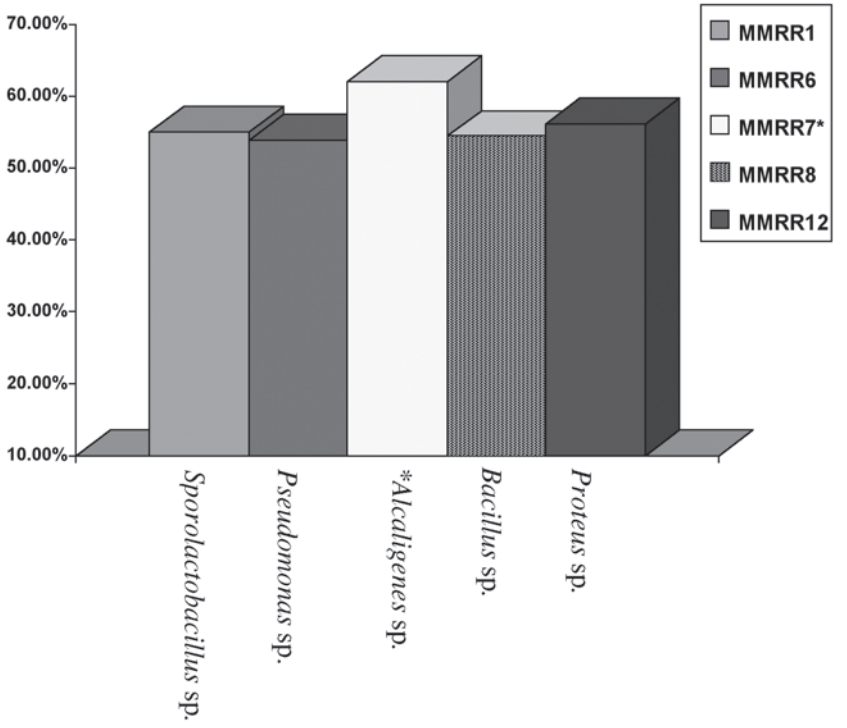


Fig. 1. Selection of potential strain for treatment of dairy wastewater.

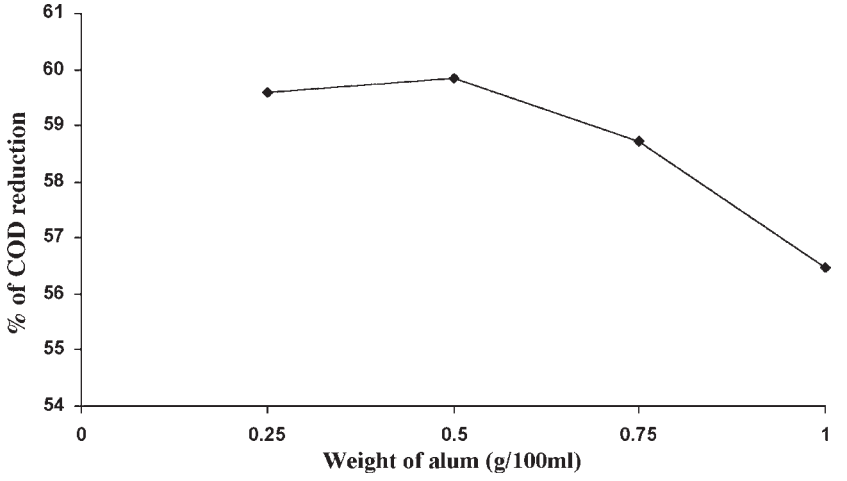


Fig. 2. Optimization of concentration of alum for primary treatment of dairy wastewater.

tion. However, the reduction was enhanced only up to 45% even after 72 h. After 96 h of treatment, the COD was reduced to 86%, and after that it remained almost constant, at about 91%, even after 144 h of treatment (Fig. 3). Comparison of the growth curve of the strain *Alcaligenes* sp. $MMRR_7$ with

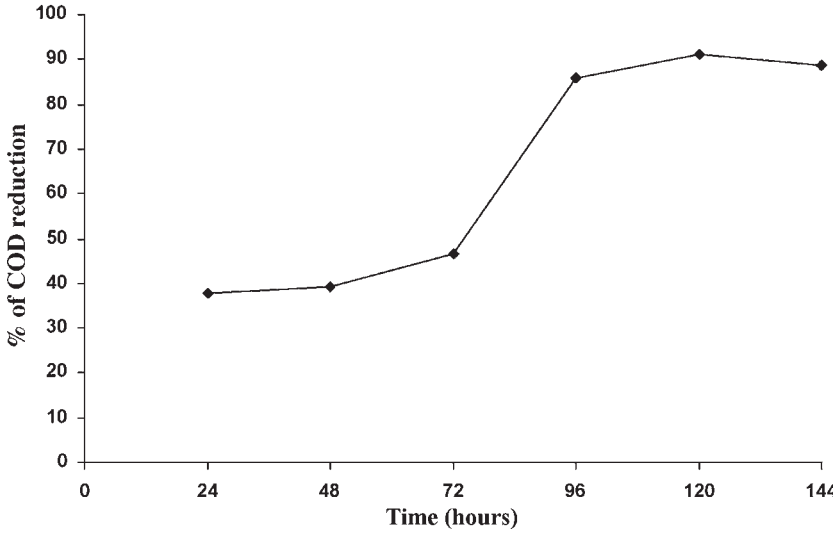


Fig. 3. Treatment of dairy wastewater with free cells of selected strain *Alcaligenes* sp. $MMRR_7$.

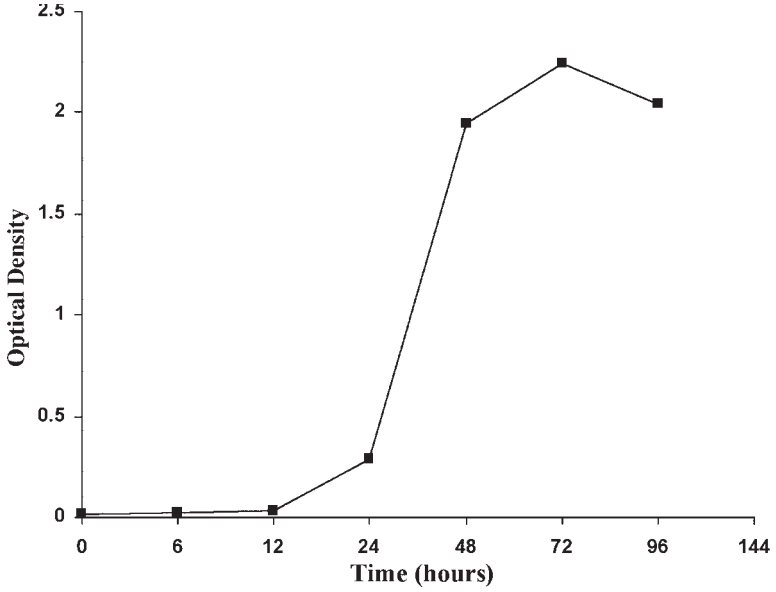


Fig. 4. Growth curve of selected strain *Alcaligenes* sp. $MMRR_7$ in dairy wastewater.

its performance during the free-cell treatment of the wastewater demonstrates that the COD reduction started during the logarithmic phase of the growth and that the maximum COD reduction was attained during the late stationary phase of growth (Fig. 4). Hence, the COD reduction is also growth associated. The present study clearly indicates the scope of treating dairy wastewater with free cells of *Alcaligenes* sp.

In a previous study conducted by Sammaiah et al. *(13)*, it was also possible to reduce the COD of the dairy wastewater by 62.8–91.3% through an upflow anaerobic filter process at a residence time of 25 d. However, in the present study one of the significant observations was that a COD reduction of >80% was possible within 4 d of residence time, and the organism maintained the same efficiency of COD reduction (80–90%) even up to 6 d of incubation. Because the rate of wastewater generation is very high in the dairy industry, a shorter treatment time will be an added advantage for the large-scale treatment of wastewater.

There are only limited reports on the treatment of dairy wastewater using a single, specific microorganism. However, Trivedy and Pattanshetty *(8)* used water hyacinth for the treatment of dairy wastewater, which resulted in 80% COD reduction during 4 d of incubation. In the present study, pretreatment of the wastewater was also found to be favorable for the enhancement of COD reduction.

In the present study, the organism isolated from the wastewater was used for treatment of dairy wastewater. This is often very helpful because the selected organism is already acclimatized to the system and can hence exhibit better efficiency *(14)*. In addition, the selected bacterial species *Alcaligenes* is well known for the ability to degrade a wide spectrum of organic compounds. Once appropriate technology is developed, *Alcaligenes* sp. can be used for large-scale treatment of dairy wastewater.

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