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# ASSESSMENT OF PHYSICO-CHEMICAL PARAMETERS OF DAIRY WASTE WATER AND ISOLATION AND CHARACTERIZATION OF BACTERIAL STRAINS IN TERMS OF COD REDUCTION

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**Abstract:** Dairy industry is a large scale food production industry and plays an important role in causing water pollution. Waste water coming out from dairy industry is categorized as raw waste and activated sludge which have to be treated by taking various parameters under consideration. This is achieved by assessment of several physico-chemical parameters of dairy waste water with raw waste which includes determination of pH, temperature, acidity, alkalinity, total dissolved solids (TDS), chemical oxygen demand (COD) out of which COD is the most important parameter. The bacterial isolation was done with activated sludge and then the raw waste was treated with the isolates to observe reduction in COD level. Three bacterial strains were isolated from dairy waste water of which two strains namely *Neisseria sp*. and *Citrobacter sp*. were effective in reducing the level of COD and thus helping in bioremediation.

**Key words:** Dairy industry waste water, activated sludge, physico-chemical parameters, COD, *Neisseria, Citrobacter,* bioremediation.

## Introduction

A dairy is a business enterprise established for the harvesting of animal milk- mostly from cows or goats, but also from buffalo, sheep, horses, or camels for human consumption. Dairy waste water is released into the environment and is hazardous to human, flora and fauna. Most of the dairy plants in the Government, Cooperatives and Private Sector produce almost similar dairy products like varieties of milk, butter, ghee, skimmed milk powder and whole milk powder (Karmakar and Banerjee, 2006). Individuals that consume low-fat dairy products are more likely to have lower weight, lower blood pressure and decreased risk of stroke, colon cancer and osteoporosis (Zemel, 2004). Some dairy products may cause health issues for individuals who have lactose intolerance and milk allergies. Some dairy products such as blue cheese may become contaminated with the fungus *Aspergillus fumigatus* during ripening, which can trigger asthma and other respiratory problems in susceptible individuals.

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Dairy products if consumed after the expiry date can cause serious heart problems (Marija, *et al*., 2009).

Dairy wastewaters are characterized by high biological-oxygen demand (BOD) and chemical oxygen demand (COD) concentrations, and generally contain fats, nutrients, lactose, detergents, sanitizing agents as well as milk constituents such as casein, lactose, fat, inorganic salts. It is estimated that about 2% of total milk processed is wasted into drains (Munavalli and Saler, 2009, Kolhe *et al.,* 2009). The wastewaters generated from milk processing can be separated into two groups—the first group comprises wastewater having high flow rates and the second comprises the effluents produced in small milk-transformation units (Castillo *et al.,* 2007). The total COD of dairy wastewater is mainly influenced by the milk, cream, or whey (Wildbrett, 1988). The pH varies in the range of 4.7–11 whereas the concentration of suspended solids (SS) varies in the range of 0.024–4.5 g/l (Passeggi *et al*., 2009). Dairy wastewaters are generally treated using biological methods such as activated sludge process, aerated lagoons, trickling filters, sequencing batch reactor (SBR), anaerobic sludge blanket (UASB) reactor, anaerobic filters (Demirel et al.,2005). In the present study, instead of these biological methods the dairy effluent was treated with different concentrations of bacteria (1% and 5%) in order to get better results.

**Materials and Methods**

**1. Sample collection**

Samples (raw waste and activated sludge) were collected from Saras dairy effluent treatment plant, Jaipur.

**2. Microbiological characterization**

The Microbial characterization was carried out by serial dilution followed by plating on nutrient media. Based on their morphological and biochemical characteristics, the isolated cultures were grouped into various genera as outlined in the *Bergey's Manual of Determinative Bacteriology* (Buchanan *et.al,* 1975). The bacterial isolation was done from activated sludge.

**3. Biochemical characterization**

The biochemical analysis of isolated cultures was done by following *Cappuccino and Sherman,* 2002. The isolated strains were then grown on differential media.

**4. Assessment of physico-chemical parameters**

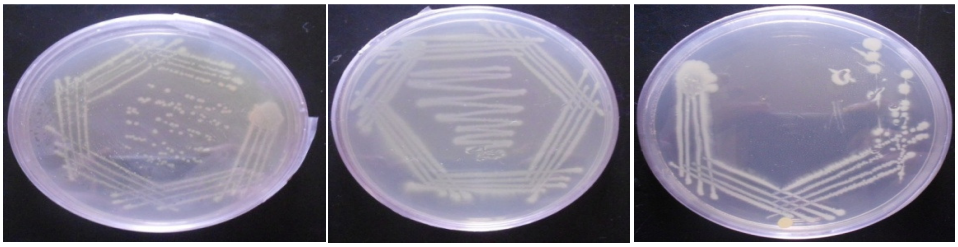
Physicochemical parameters of dairy waste water were assessed using raw effluent as sample following APHA, 1995.

### 5. COD determination after bacterial inoculations

The dairy effluent was treated with 1% and 5% bacterial inoculums in order to determine its COD reduction. COD was recorded regularly for next 5 days along with other physical parameters.

## Results and Discussion

### Microbiological Characterization



S1 S2 S3

**Fig.1** Quadrant streaking plates of bacterial isolates *(S1, S2, S3 represents strain1, strain2, strain3 respectively)

After quadrant streaking the presence of bacterial strains was confirmed by several biochemical tests.

| S. no | Gram staining | Shape | Biochemical tests | | | | | | | Partial identification |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Glucose | Lactose | Nitrate reduction | Indole production | Methyl red | Citrate utilization | $H_2S$ production | |
| S1 | - | Rod | + | NA | + | NA | NA | NA | NA | *Neisseria sp.* |
| S2 | - | Rod | NA | + | NA | - | + | NA | - | *Klebsiella sp.* |
| S3 | - | Cocci | NA | + | NA | + | NA | + | NA | *Citrobacter sp.* |

**Table 1:** List of bacterial isolates along with their biochemical tests and Gram staining results

### Assessment of physico-chemical parameters

All the physico- chemical parameters were studied immediately after the sample collection. $P^H$ and temperature were measured at the site of sampling.

| S. No | Parameters | Results |
|---|---|---|
| 1. | $p^H$ | 8.5 |
| 2. | Temperature | $33^0$c |
| 3. | Alkalinity | 140mg/ml |

| | | |
|---|---|---|
| 4. | Acidity | 97.5mg/ml |
| 5. | Total dissolved solids (TDS) | 132mg/ml |
| 6. | Chemical oxygen demand (COD) | 7100mg/l |

**Table2:** The physico- chemical analysis of raw effluent from dairy plant

**5 days observation of dairy effluent with 1% and 5% bacterial inoculums in terms of COD reduction**

The graphical representation (fig. 2 and 3) shows the COD values that are observed during 5 days treatment of the effluent. From the graphs it is very clear that the COD of the effluent is continuously decreasing and it indicates that the strains used for treating the effluent are helpful in COD reduction. The COD reduction percentage was calculated by using following formula-

%COD reduction= COD value initial-COD value final/initial COD valuex100

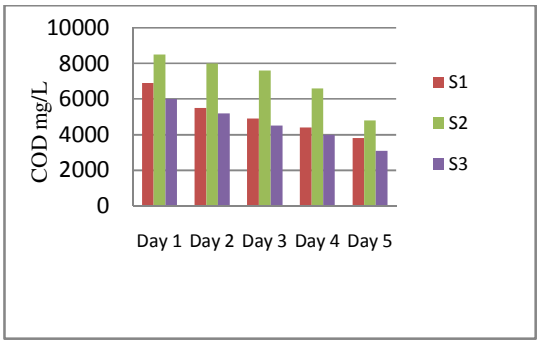



**Fig.2** Using 1% bacterial inoculums

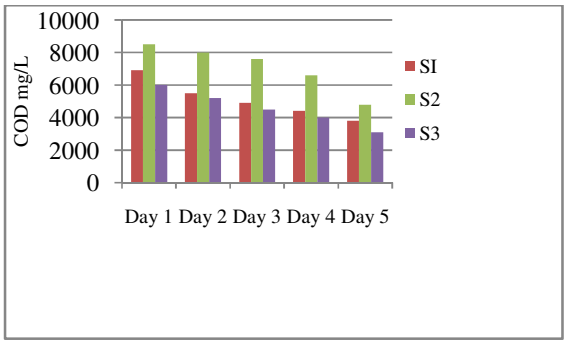



**Fig. 3** Using 5% inoculums

The treatment of dairy effluent for 5 days with 1% bacterial inoculum indicates that the maximum reduction in COD level was observed with S1 and the level reduced to 67.1% but with 5% the level is reduced to 44.9%. The maximum reduction in COD level with 5% inoculum was observed with S3 and the level reduced to 48.3% but with 1% the level reduced to 42.3%.

**5 days observation of dairy effluent for determining O. D values**

Both the figures (4 and 5) represent the O.D. values taken at 600 nm during 5 days treatment of the effluent. The O.D. of the effluent is continuously increasing which indicates an increase in bacterial cell mass.

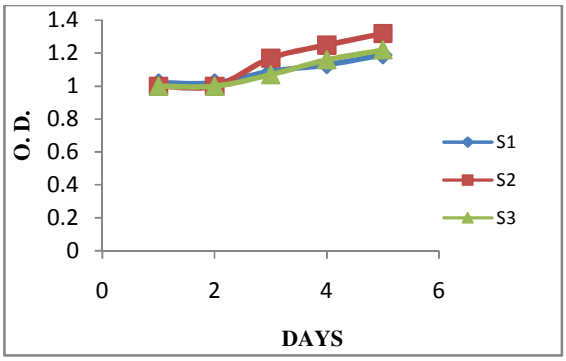



**Fig. 4** Using 1% inoculum

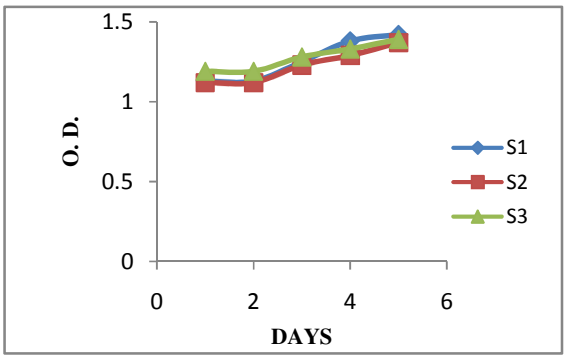



**Fig. 5** Using 5% inoculum

**5 days observation of dairy effluent pH values**

During 5 days treatment of the dairy effluent it has been observed that there is a minor fluctuation in pH values. No major change takes place.

| DAYS | SI inoculum | | S2 inoculum | | S3 inoculum | |
|---|---|---|---|---|---|---|
| | 1% | 5% | 1% | 5% | 1% | 5% |
| Day1 | 7.5 | 7.5 | 8.5 | 8.5 | 8.5 | 7.8 |
| Day2 | 7.5 | 7.5 | 8.5 | 8.5 | 8.5 | 7.8 |
| Day3 | 6.5 | 7.5 | 8.0 | 8.5 | 7.5 | 7.5 |
| Day4 | 6.5 | 7.2 | 7.0 | 8.0 | 7.2 | 7.0 |
| Day5 | 6.5 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 |

**5 days observation of dairy effluent temperature**

During 5 Days treatment of the dairy effluent it has been observed that there is a minor fluctuation in the temperature of the treated effluent.

| DAYS | S1 inoculum (1% & 5%) | S2 inoculum (1% & 5%) | S3 inoculum (1% & 5%) |
|---|---|---|---|
| Day1 | 25 | 25 | 25 |
| Day2 | 25 | 25 | 25 |
| Day3 | 24 | 24 | 24 |
| Day4 | 24 | 24 | 24 |
| Day5 | 24 | 24 | 24 |

## Conclusion

In the present study, the bacterial isolates from effluent water were used for treatment of dairy waste water. The partial identification of all the three isolates were done according to *Bergey's Manual of Determinative Bacteriology* and they are shown to be *Niesseria sp., Citrobacter sp., Klebsiella sp. Niesseria* are Gram negetive proteobacteria, *Citrobacter* is a genus of Gram negetive coliform and *Klebsiella* are Gram negative rod shaped bacteria. All the three isolates were effectively reducing the COD level but the most suitable bacterial species are *Niesseria sp.* and *Citrobacter sp.*as they reduces the COD level to 67.1% and 48.3% respectively. Both the species are well known to degrade organic compound such as nitrate.

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