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Performance Evaluation of Two Stage Wastewater Model for Treatment of Dairy Effluents

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ABSTRACT

The continuous rise in consumer demand for dairy products has led to significant advancements in food and dairy technology, resulting in a substantial increase in dairy production. However, the expansion of the dairy industry has placed a strain on the environment due to the generation of waste products containing elevated levels of constituents such as casein, lactose, fat, inorganic salts, as well as detergents and sanitizers. These contribute to elevated Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) values. While dairy facilities have implemented their own treatment plants to address these environmental challenges, there is an ongoing need to assess the efficiency of these wastewater treatment systems.

In this study, a two-stage model was developed, comprising a primary tank involving microbial treatment and a secondary tank with a layer of activated charcoal powder at the bottom and sawdust above it. The treated dairy effluent from these filtration units underwent analysis for various physicochemical parameters and was compared with samples obtained directly from the dairy industry. A total of eight indigenous bacterial isolates were derived from the processing of dairy wastewater through the model. Isolates R2 and R3 were obtained from raw wastewater, S1, S2, S3 isolates from secondary treated effluent, and T1, T2, T3 isolates from tertiary treated effluent. These isolates were further characterized biochemically as *Micrococcus* spp., *Staphylococcus aureus, Bacillus* spp., *Staphylococcus* spp., etc. Among all isolates, S3 demonstrated the highest reduction efficiency in water parameters, namely Electrical Conductivity, Chemical Oxygen Demand, and Biochemical Oxygen Demand, with reductions of 59.26%, 70.19%, and 74%, respectively

Key words: Dairy wastewater, Physicochemical analysis, Bioremediation, Microbial chacterization, Effluent treatment, Sustainable wastewater treatment.

INTRODUCTION

India is one of the highest milk producers, contributing approximately 209.96 million tonnes of milk in year 2021-2022, accounting for about 24% of global milk production, as reported by the Food and Agricultural Organization Corporate Statistical Database (FAOSTAT). This significant contribution to the dairy industry has positioned India as a key player in meeting global milk demands. Rapid industrialization, fueled by the escalating population in developing countries, has led to increased pollution of natural water sources due to the unregulated discharge of industrial wastewater into water bodies (Ahmed et al. 2021).

The food sector, known for its high water consumption, has become a major contributor to environmental challenges, with the dairy industry being a substantial part of this sector. Milk processing industries play a crucial role by producing a wide range of products, including cheese, butter, yogurt, buttermilk, cream, ice cream, whey powder, and condensed milk. However, this productivity comes at a cost, as the dairy industry stands out as the largest effluent and sludge-producing sector. On average, the processing of one liter of milk generates 2.5-3 liters of wastewater. This effluent, often discharged without proper treatment, contributes to water and soil pollution, affecting the physicochemical characteristics of the surrounding environment (Bhambri et al. 2021, Biswas et al. 2019).

Despite India's remarkable achievement as the highest milk-producing country globally, the dairy industry faces challenges related to wastewater management. The discharge of untreated or partially treated wastewater with a high organic load directly into water bodies is a significant environmental concern, leading to water pollution (Das et al. 2022).

As the demand for dairy products continues to rise, addressing these environmental challenges becomes imperative for sustainable and responsible growth in the dairy industry.

Poorly treated wastewater with a high degree of pollutants resulting from poor design, operation, and treatment system creates significant environmental problems while affecting both the water quality as well as the microbial and aquatic flora (Anonymous 2005).Some of the technologies which can reduce the harmful effect of the effluent include sedimentation, screening, aeration, filtration, flotation, degasification, chlorination, ozonation, neutralization, coagulation, absorption, ion exchange, etc. These treatments reduce the harmful effects of effluent before discharging but does not significantly decreases the organic load (Healy et al. 1995).

Industrial wastewater diminishes the quality of both groundwater and water bodies, causing harm to microbial and aquatic flora and fauna and resulting in environmental degradation. Effluents from dairy industries are laden with fats, oils, and greases (FOGs), which can adversely affect wastewater treatment systems, leading to foul odors and blockages in pipes and sewer lines. The most prevalent volatile organic compounds in dairy manure are Volatile Fatty Acids (VFAs), linked to odor issues. Additionally, dairy wastewater contains substantial amounts of nitrogen and phosphorus, posing a risk of groundwater contamination (Guillen-Jimenez et al. 2000). If released into water bodies, it can contribute to eutrophication (Ahmad et al. 2019 and Kushwaha et al. 2011), proving detrimental to the entire ecosystem (Stanchev et al. 2020). Therefore, it is crucial to ensure effective treatment of dairy wastewater before its discharge into natural water resources for the holistic well-being of the ecosystem.

Thus, a more sustainable wastewater treatment technology is desirable that operates bidirectional: reducing the environmental burden on one end and efficiently diminishing the organic and inorganic load from the effluent on the other. In the present study, we have designed a two-stage bioreactor for the treatment of dairy wastewater where we hypothesize that the efficiency of bioremediation can be increased through the use of indigenous microbes isolated from dairy effluent.

The first stage of the bioreactor employs microbial treatment to harness the natural capabilities of indigenous microbes in breaking down and assimilating organic pollutants present in the wastewater. In the second stage, a physical treatment approach is implemented using natural adsorbents. This involves the use of naturally occurring substances with adsorption properties to effectively capture and remove residual contaminants, further refining the quality of the treated effluent. By combining these two treatment strategies, our bioreactor system aims to synergistically address the complex composition of dairy wastewater, ultimately achieving a more comprehensive and sustainable solution for wastewater treatment in the dairy industry.

MATERIAL AND METHODS

Sample collection (grab sampling) and analysis

The samples were collected from a Dairy effluent treatment Plant located in Jaipur City of Rajasthan. The effluent was collected from the Raw, Secondary, and Tertiary treatment sites of the ETP. Samples were transported to the laboratory under controlled and desired conditions for further analysis. The physicochemical parameters analyzed were pH, temperature, electrical conductivity, total dissolved solids, total phosphate, nitrate, alkalinity, acidity, salinity, dissolved oxygen (DO), biochemical oxygen demand (BOD), and chemical oxygen demand (COD) (Anonymous 2005).These properties get altered with the amount of pollutants entering the water.

Isolation and characterization of microorganisms

The isolation of micro flora from the influent involved a sequential dilution of the samples, followed by spreading the diluted samples onto nutrient agar plates to obtain single, isolated colonies. After an incubation of 24 hrs at 37°C, distinct colonies were morphologically characterized based on their color, shape, elevation, surface, appearance, and pigmentation and were further subjected to sub-culturing to get the pure cultures. The initial level of identification was done through Gram's Staining. Isolates were then microscopically differentiated to be gram-positive or gram-negative based on the ability of their cell walls to retain the stain. Finally the isolates were biochemically characterized through various Biochemical tests like Starch hydrolysis, Catalase test, IMViC tests, etc for evaluating their metabolic capabilities. Based on the results obtained from these tests, the bacterial species were identified using Bergey's Manuals of Systematic Bacteriology (Pawar et al. 2011).

Experimental setup

A two-stage model was set up for the practical application of the research idea for treating the dairy effluent (Fig. 1). The idea of the design was taken from the model suggested by Krishna et al. (2005), and Porwal et al. (2015), for treatment of dairy wastewater. The model was modified as per the requirements.

Two plastic bins having a capacity of 7L each were used for making two separate columns (considered primary and secondary tanks) in the model. They were placed on iron rings, having stands nailed onto a wooden plank for support. Holes were made at desired positions in the bins and transparent silicon pipes were connected using an adhesive. An aerator was used to provide continuous aeration so that a desired level of dissolved oxygen (above 5 mg/L) can be maintained. The primary tank is involved in the microbial treatment of the effluent which is further connected to the secondary tank through silicon pipes where activated charcoal powder and sawdust were used to form a filtration assembly. The activated charcoal powder was heated in an oven at 70°C for 2 hrs before use. Sawdust was obtained from a plywood shop located in the Timber Market of Jaipur.

RESULTS AND DISCUSSION

Physico-chemical analysis

The physico-chemical characteristics of waste water samples and the permissible limits are given in Tables 1, 2. Wastewater from various sections of dairy processing units collectively comes to the inlet of ETP, from where the raw effluent was collected for the study. The secondary and tertiary treated effluents were collected from the outlet of the treatment plant. It can be seen that parameters like salinity, acidity, alkalinity, and TDS were within ISI standards, but others like DO, BOD, COD, and nitrate were not in the permissible limits.

Table 1. Permissible standard of sewage treatment plant outputs

Parameter	Mega and metropolitan cities	Class I cities	Others	Deep marine outfall
pH	5.5 - 9.0	5.5 - 9.0	5.5 - 9.0	5.5-9.0
BOD	10	20	30	30
TSS	20	30	50	50
COD	50	100	150	150
TN	10	15	-	-
TP (For discharge into ponds, lakes)	1	1	1	
Fecal Coliform (FC) (MPN/100 ml)	Desirable - 100 Permissible - 230	Desirable - 230 Permissible -1000	Desirable - 1000 Permissible-10000	Desirable -1000 Permissible-10000

Note: (i) Mega-Metropolitan Cities have population more than 1 crore, Metropolitan Cities-Population more than 10 Lakhs and Class-1 Population more than 1 Lakh. (ii) All value in mg/l except for pH and Fecal Coliform. (iii) These standards will be applicable for discharge into water bodies as well as for land disposal/applications. (iv) These Standards shall apply to all new STPs for which construction is yet to be initiated. (v) The existing/under construction STPs shall achieve these standards within 07 years from the date of notification. (vi) In case where the marine outfall provides a minimum initial dilution of 150 times at the point of discharge and a minimum dilution of 1500 times at a point 100m away from discharge point, then norms for deep sea marine discharge shall be applied. (vii) Reuse/Recycling of treated effluent shall be encouraged. (viii) State Pollution Control Boards/Pollution Control Committees may make these norms more stringent considering the local conditions.

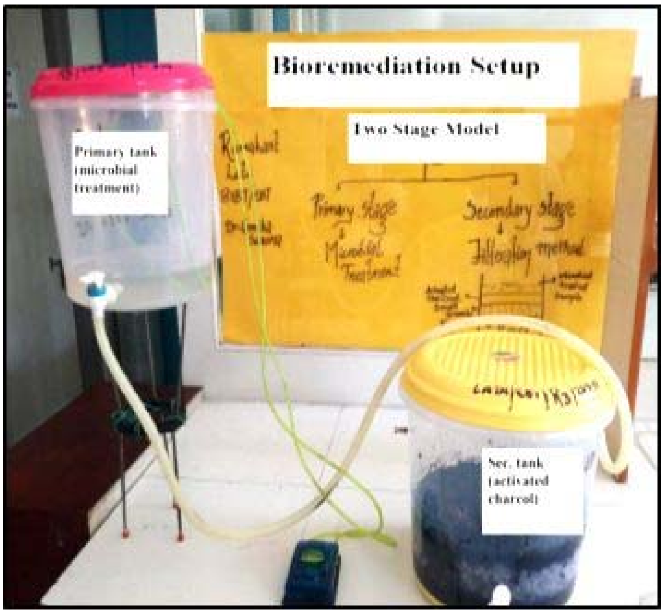

Figure 1. Experimental set-up for two stage model

Table 2. Physico-chemical characteristics of wastewater

Parameters	Raw sample	Secondary treated	Tertiary treated	ISI Standards
pH	9.39	8.26	8.15	6.5 – 8
Temperature	28.6	28.9	28.7	-
EC	2332	1836	1190	-
Salinity	2.6	3.6	2.3	-
Acidity	700	450	300	-
Alkalinity	210	256.60	100	-
TDS	666	919	594	-
DO	0.1	2.26	2.65	4 – 6
BOD	200	160	120	50
COD	520	380	256	250
Nitrate	16.28	13.49	11.64	10
Phosphate	1048.57	391.4	380	-
Sulfate	35.5	15.1	19.08	-

Characterization and identification

A total of eight bacterial isolates were obtained based on their size, shape, margin, elevation, surface, etc. (Fig. 2 and Table 3). These isolates were microscopically identified through gram staining (Fig. 3 and Table 4).

Biochemical characterization and identification

After segregating based on cellular morphology i.e., Cocci and Rod shaped morphology their biochemical identification was performed. Isolates R2, S1, T1 and T2 showed only positive catalase activity and negative activity for both mannitol fermentation and pigment production and thus identified as *Staphylococcus* spp., whereas isolate R3 and T3 showed positive results for both catalase and pigment production and thus identified as *Micrococccus* spp. Among all isolates of cocci shaped, only isolate S3 was positive for mannitol fermentation and identified as *Staphylococcus aureus*. The rod-shaped isolate, identified as *Bacillus* sp., showed positive starch utilization and simmons citrate results, indicative of its ability to metabolize starch and citrate as sole carbon sources (Fig. 4 and Table 5).

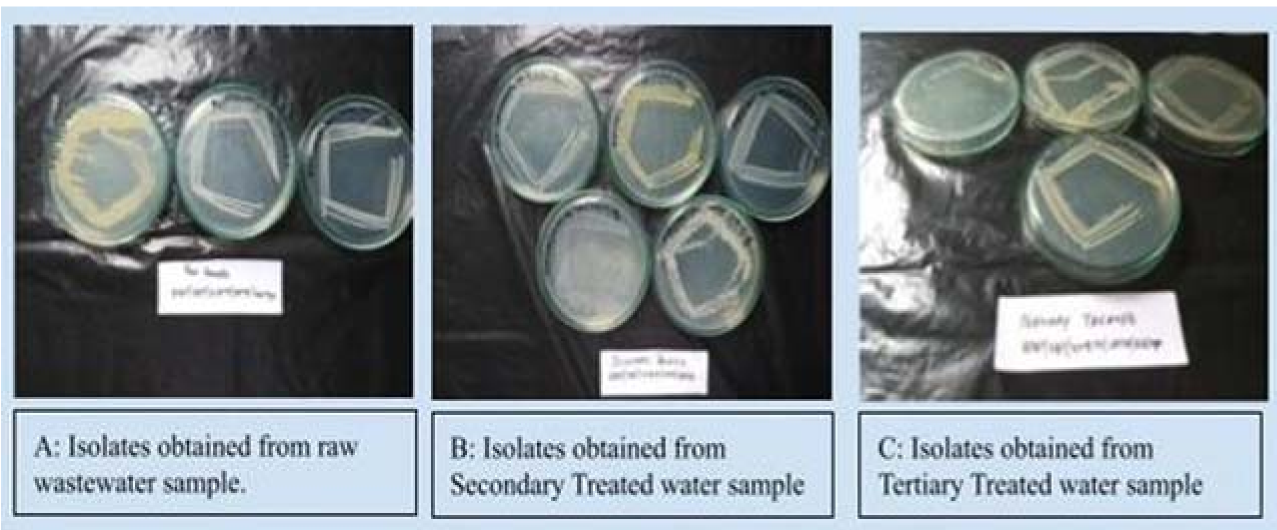

Figure 2. Isolation of bacteria from samples of raw wastewater, secondary treated and tertiary treated water

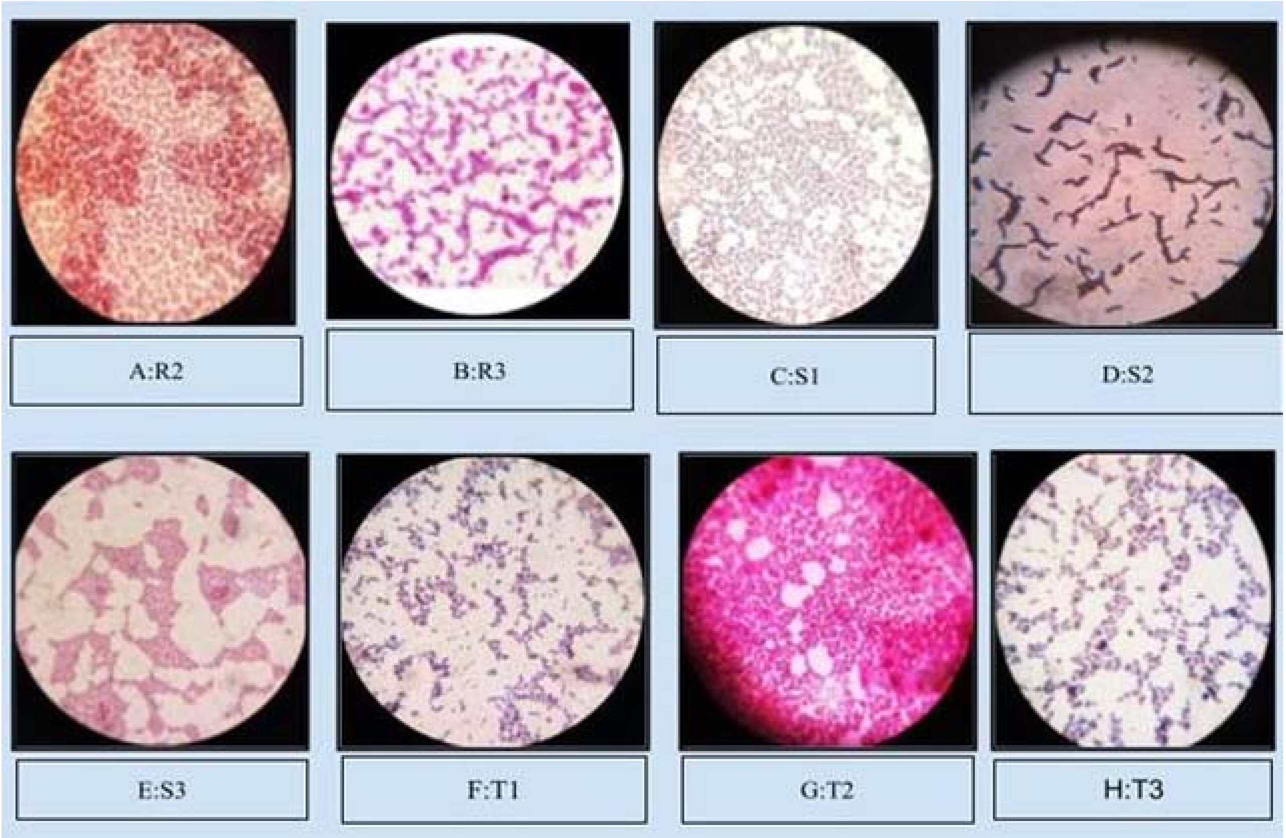

Figure 3. Gram's staining of bacterial isolates

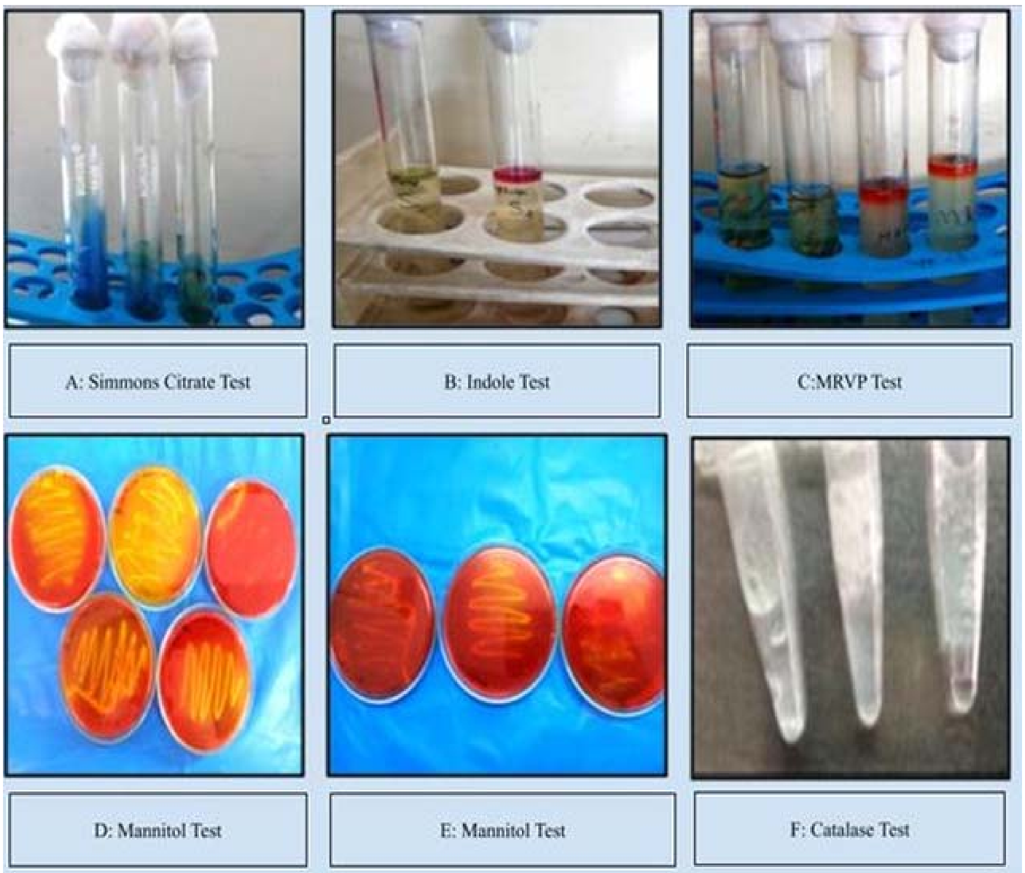

Figure 4. Biochemical test of bacterial isolates

Table 3. Morphological characterization of the bacterial isolates

Strain	Form	Elevation	Margin	Surface
R2	Irregular	Raised	Entire	Rough
R3	Punctiform	Flat	Undulate	Rough
S1	Punctiform	Raised	Undulate	Smooth
S2	Circular	Flat	Entire	Smooth
S3	Punctiform	Flat	Undulate	Rough
T1	Punctiform	Flat	Entire	Rough
T2	Irregular	Flat	Lobate	Smooth
T3	Punctiform	Flat	Undulate	Smooth

Table 4. Microscopic characterization of the bacterial isolates

Isolates	Gram's stain	Shape
R2	Gram positive	*Cocci*
R3	Gram positive	*Cocci*
S1	Gram positive	*Cocci*
S2	Gram positive	*Rod*
S3	Gram positive	*Cocci*
T1	Gram positive	*Cocci*
T2	Gram positive	*Cocci*
T3	Gram positive	*Cocci*

Bioremediation

Further these isolates were examined for their ability to reduce pollutants from dairy effluent. Bioremediation is an efficient process to reduce organic pollution from effluents of ETPs. It is possible to humiliate about 50% of the initial pollution load from the effluent. The results showed that these isolates were able to reduce COD, BOD, and other parameters effectively from dairy effluent (Table 6). It was observed that the S3 isolate showed better results as compared to other strains. Mixed culture was also prepared to treat the effluent and check its efficiency.

pH: The pH of the untreated sample was alkaline. It was variable even after microbial treatment. After filtration, the values were near to neutral. The pH of dairy wastewater depends on the nature of the end product and can vary between 6.6 to 12.2 (Munavalli and Saler 2009).

Electrical Conductivity (EC): It can be observed that after aeration, the reduction efficiency of the S3 isolate was highest showing a 59.26 % reduction (Table 6). The reduction in EC after aeration might be due to the use of ions by microorganisms for their growth and survival. Reduction after filtration was associated with the use of combined filtering agents like sawdust and activated charcoal. The important ions that impart conductivity in water are Cl-SO_4, CO_3, HCO_3 and NO_3. EC is an important water parameter that can be used for the detection of impurities in water. Quantitative estimation of ionic constituents which are important for boiler feed water and cooling water can also be detected through EC (Porwal et al. 2015).

Total suspended solids: Physical treatment ie, aeration and filtration reduce total suspended solids during the study. The S3 isolate showed a 30.78% reduction when compared to other isolates.

Table 5. Biochemical characterization of the bacterial isolates

Cocci shaped						
Isolates	**Catalase**	**Mannitol**	**Pigment**			**Species identified**
R2	+	-	-			*Staphylococcus* spp.
R3	+	-	+			*Micrococcus* spp.
S1	+	-	-			*Staphlococcus* spp.
S3	+	+				*Staphylococcus aureus*
T1	+	-	-			*Staphylococcus* spp.
T2	+	-	-			*Staphylococcus* spp.
T3	+	-	+			*Micrococcus* spp.
Rod shaped						
Isolates	**Starch**	**Indole**	**MR**	**VP**	**Simmons citrate**	**Species identified**
S2	+	-	-	-	+	*Bacillus* sp.

Table 6. Degrading capability of various isolates

Parameter	Raw water	R2	R3	S1	S2	S3	T1	T2	T3	Mix culture	ISI standard
pH	9.39	8.12	8.66	8.0	7.9	8.0	**7.7**	7.8	7.8	**6.8**	6.5-8
EC	2332	1854	1783	2823	1970	950	2711	2711	2487	2824	-
TDS	666	558	592	496	573	461	556	552	592	494	-
DO	0.1	5.21	5.10	5.22	5.32	**5.51**	5.98	5.74	5.81	5.71	4-6
Phosphate	1048.57	350.21	352.45	355.36	450.36	280.69	400.65	685.36	785.98	300.56	
Sulphate	35.5	30.11	29.14	32.45	31.24	28.75	35.61	32.64	30.25.	25.16	-
Nitrate	16.28	14.34	14.02	11.54	11.73	**9.23**	10.02	10.08	10.06	**9.01**	10
BOD	200	78	85	66	82	52	62	72	74	**58**	50
COD	520	235	300	200	198	**155**	180	215	208	175	250

Chemical Oxygen Demand (COD): COD content of effluent after aeration was significantly reduced by S3 and the mixed culture. S3 reduced COD by 70.19% whereas mixed culture showed a 66.34% reduction. The reduction in COD values is due to more amounts of dissolved organic nutrients. Our results of COD reduction are in concurrence with Guillen-Jimenez et al. (2000), where maximum COD reduction was up to 65 to 70%.

Biochemical oxygen demand (BOD): After aeration, the reduction efficiency of S3 was highest at 74% for BOD. The reduction shown by mixed culture after 48 h of aeration was second highest (71%). It was observed that R3 showed a very poor reduction efficiency of 57.5%. The dairy industry generates huge effluent characterized by high COD and BOD representing their elevated organic content such as fats, lactose, detergents, sanitizing agents, casein, and inorganic salts. Yathavamoorthi et al. (2010) measured the relationship between fecal coliforms and BOD, but not with suspended solids, and found that adsorption of fecal coliforms was more important than sedimentation. Though the high growth of microbes consumed the oxygen present in the treatment column, continuous and excess aeration proved to be an important reason for the reduction of BOD in the first treatment column. An important aspect of biological treatment includes the removal of organic matter and nutrients from the wastewater.

Sulphate: After aeration period of 48 h, all isolates showed poor reductions in sulfates (Table 6) S3 and mixed culture showed 19 and 29.1% reduction, respectively. From the results obtained, it is clear that the overall sulfate reduction efficiencies of all the isolates were quite low. A high concentration of sulfate in water causes a laxative effect when combined with calcium and magnesium, which are the most common constituents of hardness. Appreciable amounts of sulfates in water form hard scales in boilers and heat exchangers. In sewers, sulfates also cause odor and corrosion during anaerobic conditions because of their conversion into hydrogen sulfide (Al-Wasify et al. 2007).

Phosphate: All isolates showed good results for phosphate reduction. S3, mixed culture and R2 showed the highest reduction of 73.21, 71.32 and 66.6%, respectively. Similar results were also reported by Guillen-Jimenez et al. (2000).

The study was carried out to check the physicochemical parameters of the samples (raw, secondary, and tertiary treated samples) before bioremediation; the results showed that all the physicochemical parameters were exceeding the ISI standard limits. Efficient primary wastewater treatment is important, especially for the removal of fat (because the breakdown of fat is slow and difficult) suspended articles. The physicochemical parameters such as pH, Temperature, E.C, Salinity (%), Alkalinity (mg/l), Acidity (mg/l), TDS (ppm), D.O (mg/l), BOD (mg/l), COD (mg/l), Nitrate (mg/l), Phosphate (mg/l) and Sulfate (mg/l) were analyzed.

The rapid decomposition and reduction of dissolved oxygen levels in the effluent not only lead to anaerobic conditions and the emission of noxious odors but also contribute to significant public health concerns. The associated problems, including the release of foul odors, have been linked to the spread of diseases such as yellow fever, chicken guinea, malaria, dengue, and diarrhea (Kumar and Desai 2011). Furthermore, the high proportion of readily degradable organic compounds present in the dairy waste and low nutrients make dairy waste favorable for the growth of microorganisms, to reduce this problem bioremediation of dairy samples was done to increase the efficiency of the treatment, for which indigenous microbes and natural adsorbent was used (Porwal et al. 2015). After bioremediation of the raw sample by 8 strains the reduction in physicochemical parameters were analyzed. The microbial isolates, particularly the S3 strain, demonstrated significant reductions in COD, BOD, and other parameters after aeration and filtration. The inclusion of activated charcoal powder and sawdust in the filtration assembly played a crucial role in achieving positive outcomes, highlighting the importance of natural adsorbents in wastewater treatment.

CONCLUSION

India has become a major contributor to global milk production due to rapid growth in the development of the dairy industry. But this substantial increase in dairy sector has led to increased pollution of natural water sources due to the unregulated discharge of untreated or partially treated wastewater

The study highlights the inherent environmental risks associated with the dairy industry, emphasizing the need for effective wastewater management strategies. The discharge of untreated effluent containing a high organic load poses a significant threat to water bodies, leading to pollution and degradation of the surrounding ecosystem. The presence of fats, oils, greases, volatile organic compounds, nitrogen, and phosphorus in dairy wastewater further complicates the environmental impact, posing risks of foul odors, blockages in pipes, and potential groundwater contamination.

To address these challenges, the research proposes a two-stage bioreactor model for the treatment of dairy effluent. This innovative approach combines microbial treatment in the first stage, harnessing the natural capabilities of indigenous microbes to break down organic pollutants, with a physical treatment approach in the second stage, using natural adsorbents to capture and remove residual contaminants. The synergistic application of these two treatment strategies aims to provide a comprehensive and sustainable solution for the complex composition of dairy wastewater.

The current finding suggests that that a more sustainable wastewater treatment technology is essential for the dairy industry. The proposed bioreactor model offers a promising avenue for addressing the environmental challenges associated with dairy wastewater, providing a cost-effective and efficient system. The present study concludes with a call for further research to explore the exact mechanisms behind bioremediation, aiming to enhance the overall efficiency of wastewater treatment systems and contribute to the responsible and sustainable growth of the dairy industry.

Authors' contributions: All authors contributed equally

Conflict of interest: Authors declare no conflict of interest

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