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# ISOLATION AND IDENTIFICATION OF MICROBIAL CONSORTIA FOR BIODEGRADABILITY OF DAIRY EFFLUENT

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**ABSTRACT:** In developing countries like India, the major part of the dairy sector is under the coverage area of the unorganized sector, which lacks adequate treatment facility. In the present investigation, the study was done to isolate most frequently occurring active strains adapted to the wastewater physical-chemical conditions and having good biodegradation potential. The three isolates were selected based on their efficiency in reducing all the three pollution potential parameters, i.e. BOD, TSS, and Oil and grease content. The identification of selected strains was done by 16 S rRNA sequencing. The maximum reduction in BOD3 was shown by isolate VDLL5, i.e. 88.5%. Isolate HDUL4 and HDUL3 were efficient in reducing the TSS content by 82.8% and 82.4%. Isolate no. VDLL6 and VDLL7 were more efficient in reducing the oil and grease content by 82.8% and 82.6% respectively.

**KEYWORDS:** Dairy wastewater, Microbiological characterization, Biodegradability, Bioaugmentation, Biological oxygen demand.

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## 1.INTRODUCTION

Dairy wastewater disposal represents a major environmental problem. Numerous effective attempts have been made to resolve this problem by the activated sludge process is the most used biological treatment ever [1]. This review discusses microorganisms associated with microbial digestion of dairy wastewater, the biochemistry of the process, factors affecting microbial digestion, and efforts to develop defined cultures. To get an efficient biological wastewater treatment, it is very important to know the wastewater microbiota composition and the biochemical properties correlated to the origin of pollutants, as well as the optimum metabolic activity and the physical-chemical conditions

[2-4]. Microbial digestion of dairy food wastewater offers many advantages over other treatments in that a high level of waste stabilization is achieved with much lower levels of sludge. As microbial digesters become increasingly used in dairy plants, more research should be directed toward selecting the best cultures that maximize environmental problem from dairy waste. The microbes responsible for the organic and inorganic luxury uptake occurring in the treatment plant [5]. The isolation of bacteria and the study of their identification have been hampered by the unreliability of conventional microbiological techniques. This is largely due to their morphological variations and inconsistent characteristics and different biochemical Characteristics [6]. To fully understand their role in promoting an activated sludge process, bacteria need to be characterized. In India, about 85% of the dairy sector is under the coverage of the unorganized sector, which lacks adequate treatment facility and management skills. Physico-chemical characteristics of the dairy wastewater generated by organized and unorganized sector exhibit huge variations. The wastewater generated by the unorganized sector is rich in organic content. Its C: N ratio was calculated as 37.6 compared to the ratio of 11.9 of the organized sector [7-11]. Variations in major pollution parameters of dairy effluent of both sectors require appropriate treatment approaches for its safe disposal. The present investigation was carried out to isolate the most frequently occurring and optimally performing microorganisms from dairy wastewater and sludge samples of unorganized sector and to test the bioremediation efficacy of the isolates by bioaugmenting them in dairy wastewater.

## 2. MATERIALS AND METHODS

### Sample collection

Three samples of dairy wastewater and three samples of dairy sludge were collected from various dairy industry located in the district of Telangana, India. In dry plastic bottles which were rinsed with distilled water and then with dairy effluent. The sample was transferred to the laboratory immediately and stored at 4 °C to avoid any physical-chemical changes in the wastewater. Three samples of dairy wastewater and three samples of dairy sludge were collected from various dairy industry located in the district of Telangana, India.

### Analysis of the dairy sludge samples

Parameters of dairy waste samples analyzed included pH, color, temperature. BOD3, Oil and grease and TSS (Total suspended solids) which were carried out as per the standard procedure. Total suspended solids were determined by the equation TSS = TS (Total solids – TDS (Total dissolved solids). The Oil and grease content was determined by partition gravimetric method. The BOD was analyzed by the titrimetric method [12].

### Isolation of most frequently occurring micro-organisms from dairy sludge

Appropriately dilute sludge samples were plated onto Nutrient Agar plates, which were then incubated at 37 °C for 24 h. After incubation, the pure cultures of the most frequently encountered isolates were prepared and used in the study. Each of the isolates was observed for the colony

characters like size, shape, color, margin, elevation, and opacity and also morphological characters like Gram reaction, shape, and arrangement of cells. Total of 50 isolates was obtained, and they were designated as 1, 2, 3……..50.

**Bioaugmentation of dairy sludge by using isolates**

To obtain greater cell biomass, a pure culture of isolates were inoculated in Nutrient Broth incubated at 37□C for 24 h. Centrifugation was done at 5000 rpm. 0.1% (w/v) wet weight basis of inoculum was used in 100 ml of dairy wastewater and incubated for 3 days in BOD incubator at 27 °C. Control was run simultaneously without bioaugmentation. The ability of the isolates to reduce BOD3, TSS, and Oil & grease was examined.

**Identification of bacterial isolates**

Extraction of DNA from bacterial isolates was done as per the protocol described by Atashpaz et al. [13]. A single colony was inoculated in nutrient broth and was grown for 24 h at 37 °C. From the 5 ml of culture, the cells were harvested. 800 µL of lysing buffer (2% CTAB, 100 mM Tris-HCl, 1.4 M NaCl, 1% PVP, 20 mM Na2EDTA and 0.2% LiCl) was added to the sample and incubated at 65 °C (30 min for Gram-negative bacteria; 2 h for Gram-positive bacteria). The sample was centrifuged at 10000 rpm for 5 min at 4 °C. After the extraction of supernatant an equal volume of chloroform-isoamyl alcohol (24:1 v/v) was added to it and was centrifuged at 12000 rpm for 8 min at 4 °C. The DNA was extracted from the aqueous layer by adding cold (−20 °C) isopropanol. The dried DNA pellet was dissolved in 50 µL of 1X TE buffer. The quality and intactness of the extracted DNA were checked by running on 1% agarose gel, which contains 1 µg/ml ethidium bromide. The A260/A280 absorbance ratio was used to determine undesired contaminations.

**PCR amplification and sequencing of 16 S rRNA gene**

PCR amplification and sequencing of the extracted DNA samples was done at Sri Yuva Biotech PVT LTD, Hyderabad. Amplification of 16 S rRNA universal primers gene fragment was done by using MJ Research Peltier Thermal Cycler. The universal primers (Forward primer 27 F AGAGTTTGATCMTGGCTCAG and Reverse primer 1492 R TACGGYTACCTTGTTACGACTT) were used.

1 µL of template DNA was added in 20 µL of PCR reaction solution. The PCR reaction was performed with the following conditions: Initial denaturation was done at 94 °C for 2 min, followed by 35 amplification cycles at 94 °C for 45 s, the annealing temperature of primers was 55 °C for 60 s, and extension at 72 °C for 60 s. The final extension was done at 72 °C for 10 min. The resulting PCR products were purified using Montage PCR Clean-up kit (Millipore) and sequenced using ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems).

**Bioinformatics protocol**

The 16 S rRNA sequence was compared using NCBI blast similarity search tool. For multiple alignments of sequences, MUSCLE 3.7 program was used [14]. Further, the program Gblocks 0.91b was used to cure the poorly aligned regions (removes alignment noise) [15]. Finally, the program PhyML 3.0 aLRT was used for phylogeny analysis, and HKY85 was used for the substitution model. The program Tree Dyn 198.3 was used for tree rendering [16].

## 3. RESULTS AND DISCUSSION

The physical parameters like temperature, pH, odor, and color of the dairy wastewater and sludge samples was recorded at the site of collection. The temperature varied from 10 to 35 °C. It was due to the seasonal variations and effects, the chemical and biological reactions taking place in water [17]. The pH of the samples varied between 3.9 and 9.8. Acidic nature of the effluent generated was mainly due to the production of cheese in the unorganized sector. The odor was always unpleasant due to anaerobic decomposition of organic matter. Color of the dairy wastewater was pale white, and the sludge samples were grey colored with large flocs of suspended matter.

**Isolation of most frequently occurring microorganisms from dairy sludge samples**

Various studies were done by many workers on the microbiological and biochemical characterization dairy wastewater of organized sector [18-20]. Whereas, only limited information is available about the bacterial diversity of unorganized sector dairy effluent and dairy sludge. This study aimed to isolate most frequently occurring and optimally performing microbial isolates from the unorganized dairy sector wastewater and the activated sludge. Total of 50 bacterial isolates was isolated and were designated as 1,2……50.

**Characterization of the bacterial isolates**

Colony and morphological characteristics of the isolates were studied.

**Colony characteristics**

Observations about colony characteristics of the isolates were presented in Table 1. The colonies of the isolates were circular to irregular. The color of colonies was generally pale white. The shape varied from regular to irregular with entire to undulate margins. The bacterial isolates were stained to observe their morphological characters, and the observations are presented in Table 1. Out of the 50 isolates isolated from dairy sludge fifteen strains were Gram-negative and these were rods, cocci, and coccobacilli. The arrangement of most of the cells was in pairs and chains. Twenty strains were found to be Gram-positive with coccobacilli morphological character. These cells were mostly present in pairs.

**Table 1: Colony and morphological characteristics of bacterial isolates**

| S.NO | Source of sample | Isolate number | Shape | Size | Margin | Elevation | color | Consistency appearance |
|---|---|---|---|---|---|---|---|---|
| **1** | Vijaya Dairy soil | VDLS1 | Circular | Small | Entire | Raised | White | Dry |
| **2** | Vijaya Dairy soil | VDLS2 | Irregular | Small | Serrate | Flat | White | Dry |
| **3** | Vijaya Dairy soil | VDLS3 | Circular | Medium | Entire | Flat | Light orange | Dry |
| **4** | Vijaya Dairy soil | VDLS4 | Circular | Small | Entire | Raised | Pale yellow | Dry |
| **5** | Vijaya Dairy soil | VDLS5 | Irregular | medium | serrate | Flat | White | Dry |
| **6** | Vijaya Dairy soil | VDLS6 | Irregular | Small | Serrate | Flat | White | dry |
| **7** | Vijaya Dairy soil | VDLS7 | Circular | Small | Entire | Raised | White | Dry |
| **8** | Vijaya Dairy soil | VDLS8 | Irregular | Medium | Serrate | Flat | White | Dry |
| **9** | Vijaya Dairy liquid | VDLL1 | Irregular | Medium | Serrate | Flat | Transparent | Sticky |
| **10** | Vijaya Dairy liquid | VDLL2 | Irregular | Medium | Serrate | Flat | White | Dry |
| **11** | Vijaya Dairy liquid | VDLL3 | Circular | Small | Entire | Flat | White | Sticky |
| **12** | Vijaya Dairy liquid | VDLL4 | Circular | Medium | Entire | Raised | White | |
| **13** | Vijaya Dairy liquid | VDLL5 | Circular | Small | Entire | Raised | White | Dry |
| **14** | Vijaya Dairy liquid | DLL6 | Irregular | Small | Serrate | Flat | White | Dry |
| **15** | Vijaya Dairy liquid | VDLL7 | Circular | Medium | Entire | Flat | Light orange | Dry |
| **16** | Vijaya Dairy liquid | VDLL8 | Circular | Small | Entire | Raised | Pale white | Dry |
| **17** | Vijaya Dairy liquid | VDLL9 | Irregular | medium | serrate | Flat | White | Dry |
| **18** | Heritage Dairy soil | HDUS1 | Irregular | Small | Serrate | Flat | White | Dry |
| **19** | Heritage Dairy soil | HDUS2 | Circular | Small | Entire | Raised | White | Dry |
| **20** | Heritage Dairy soil | HDUS3 | Irregular | Medium | Serrate | Flat | White | Dry |
| **21** | Heritage Dairy soil | HDUS4 | Irregular | Medium | Serrate | Flat | Transparent | Sticky |
| **22** | Heritage Dairy soil | HDUS5 | Irregular | Medium | Serrate | Flat | White | Dry |
| **23** | Heritage Dairy soil | HDUS6 | Circular | Small | Entire | Flat | White | Sticky |
| **24** | Heritage Dairy soil | HDUS7 | Circular | Medium | Entire | Raised | White | Sticky |
| 25 | Heritage Dairy soil | HDUS8 | Irregular | Small | Serrate | Flat | White | Dry |
| **26** | Heritage Dairyliquid | HDUL1 | Circular | Small | Entire | Raised | Pale yellow | Dry |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **27** | Heritage Dairy liquid | HDUL2 | Irregular | Medium | Serrate | Flat | White | Dry |
| **28** | Heritage Dairy liquid | HDUL3 | Irregular | Medium | Serrate | Flat | White | Sticky |
| **29** | Heritage Dairy liquid | HDUL4 | Irregular | Medium | Serrate | Flat | White | Dry |
| **30** | Heritage Dairy liquid | HDUL5 | Circular | Small | Entire | Flat | White | Sticky |
| **31** | Heritage Dairy liquid | HDUL6 | Circular | Medium | Entire | Raised | Transparent | Sticky |
| **32** | Heritage Dairy liquid | HDUL7 | Irregular | Small | Serrate | Flat | White | Dry |
| **33** | Heritage Dairy soil | HDNS1 | Circular | Medium | Entire | Raised | White | Dry |
| **34** | Heritage Dairy soil | HDNS2 | Irregular | Medium | Serrate | Flat | White | Dry |
| **35** | Heritage Dairy soil | HDNS3 | Irregular | Medium | Serrate | Flat | White | Sticky |
| **36** | Heritage Dairy soil | HDNS4 | Irregular | Medium | Serrate | Flat | White | Dry |
| **37** | Heritage Dairy soil | HDNS5 | Circular | Small | Entire | Flat | Pale white | Sticky |
| **38** | Heritage Dairy soil | HDNS6 | Circular | Medium | Entire | Raised | White | Sticky |
| **39** | Heritage Dairy soil | HDNS7 | Irregular | Small | Serrate | Flat | White | Dry |
| **40** | Heritage Dairy soil | HDNS8 | Circular | Small | Entire | Raised | White | Dry |
| **41** | Heritage Dairy soil | HDNS9 | Irregular | Medium | Serrate | Flat | White | Dry |
| **42** | Heritage Dairy liquid | HDNL1 | Irregular | Medium | Serrate | Flat | White | Sticky |
| **43** | Heritage Dairy liquid | HDNL2 | Irregular | Medium | Serrate | Flat | Pale white | Dry |
| **44** | Heritage Dairy liquid | HDNL3 | Circular | Small | Entire | Flat | White | Sticky |
| **45** | Heritage Dairy liquid | HDNL4 | Circular | Medium | Entire | Raised | White | Dry |
| **46** | Heritage Dairy liquid | HDNL5 | Irregular | Small | Serrate | Flat | White | Dry |
| **47** | Heritage Dairy liquid | HDNL6 | Circular | Medium | Entire | Raised | White | Dry |
| **48** | Heritage Dairy liquid | HDNL7 | Irregular | Medium | Serrate | Flat | White | Dry |
| **49** | Heritage Dairy liquid | HDNL8 | Irregular | Medium | Serrate | Flat | White | Sticky |
| **50** | Heritage Dairy liquid | HDNL9 | Circular | Medium | Entire | Raised | White | Dry |

Among 50 isolates, obtained from dairy sludge samples, Gram-negative character were exhibited by fifteen isolates. They were mainly rods. Gram-positive character was exhibited by thirty-five isolates. These were rods and coccobacilli. The cells were present singly, pairs, in chains and clusters. The microscopic characteristics of the ten most efficient bacterial isolates are shown in Fig. 1.

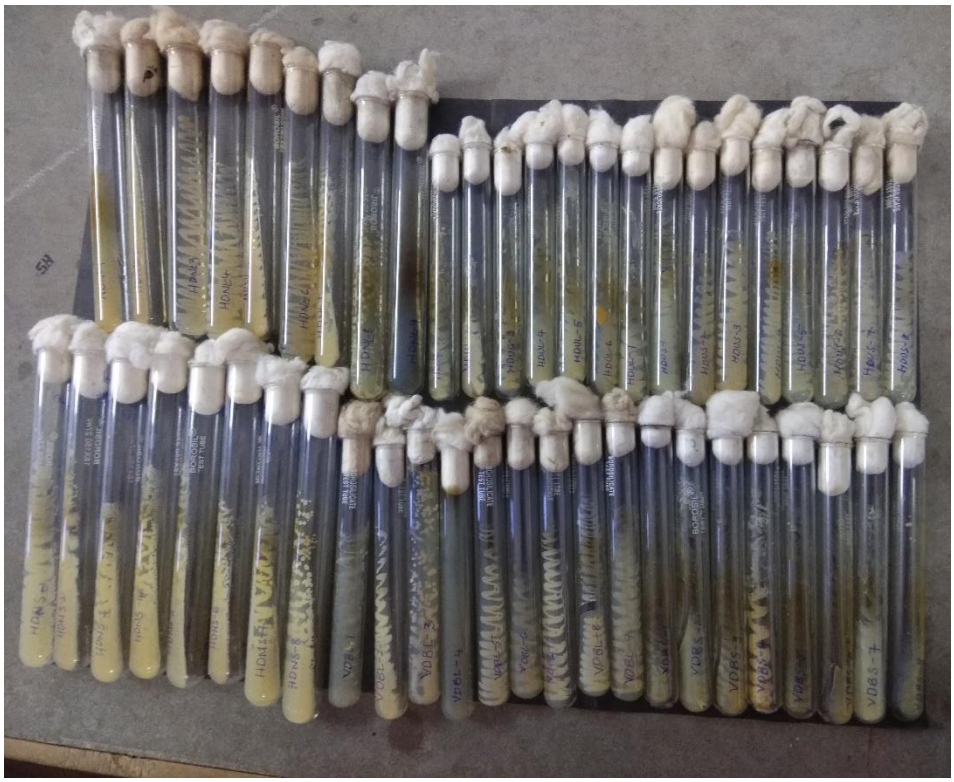

**Fig 1: Pure culture was obtained by sheaths on nutrient agar**

**pH**

The pH of untreated dairy wastewater was mainly acidic. It varied between 3.9 and 9.8. The pH of dairy effluent depends on the nature of the end product. The effluent exhibiting the acidic conditions could have a serious impact on soil and microflora [1]. Post-treatment with microbial isolates pH of dairy water was observed to be mildly acidic to alkaline.

**Bioaugmenting dairy wastewater with bacterial isolates**

Biological methods employing indigenous microflora are generally used for the treatment of dairy wastewater, but with time biodegradative ability decreases as mortality rate increases due to huge variations in the characteristics of the effluent. The bioaugmentation strategy can be used to treat the wastewater. It enhances the treatment process by introducing specifically selected strains of micro-organisms or microbial consortia to achieve desirable results [21]. 50 bacterial isolates were examined for their ability to reduce the Biological Oxygen Demand, Total Suspended Solids, and Oil and grease content. Results in Table 2 represent the percentage reduction in BOD3, TSS, and Oil and grease content by the bacterial isolates.

**Table 2: Bioremediation efficacy of dairy waste water**

| S.No | Isolate no. | pH | BOD (% Reduction) | TSS (% Reduction) | Oil and Grease (% reduction) |
|---|---|---|---|---|---|
| 1 | VDLS1 | 7.48 | 78.7 | 81.2 | 78.4 |
| 2 | VDLS2 | 7.25 | 66.4 | 52.8 | 45.8 |
| 3 | VDLS3 | 8.20 | 58.5 | 48.2 | 38.6 |
| 4 | VDLS4 | 7.30 | 73.2 | 76.4 | 78.4 |
| 5 | VDLS5 | 4.07 | 25.0 | 21.8 | 22.8 |
| 6 | VDLS6 | 8.43 | 60.0 | 48.5 | 50.8 |
| 7 | VDLS7 | 7.04 | 63.2 | 49.6 | 52.4 |
| 8 | VDLS8 | 3.92 | 18.0 | 21.3 | 24.8 |
| 9 | VDLL1 | 7.45 | 87.3 | 82.4 | 80.8 |
| 10 | VDLL2 | 8.70 | 60.0 | 54.6 | 54.2 |
| 11 | VDLL3 | 9.24 | 35.5 | 48.9 | 36.8 |
| 12 | VDLL4 | 9.87 | 40.0 | 34.6 | 26.8 |
| 13 | VDLL5 | 7.52 | 88.4 | 80.2 | 78.2 |
| 14 | VDLL6 | 7.68 | 83.2 | 78.8 | 82.8 |
| 15 | VDLL7 | 7.32 | 80.4 | 79.2 | 82.6 |
| 16 | VDLL8 | 8.10 | 66.2 | 52.4 | 62.4 |
| 17 | VDLL9 | 8.82 | 52.8 | 46.8 | 54.8 |
| 18 | HDUS1 | 5.20 | 44.8 | 34.6 | 48.4 |
| 19 | HDUS2 | 6.42 | 62.0 | 53.4 | 52.4 |
| 20 | HDUS3 | 6.80 | 72.8 | 64.8 | 52.4 |
| 21 | HDUS4 | 4.72 | 35.6 | 23.4 | 28.8 |
| 22 | HDUS5 | 4.17 | 28.0 | 28.8 | 22.4 |
| 23 | HDUS6 | 7.28 | 76.0 | 68.9 | 78.8 |
| 24 | HDUS7 | 9.21 | 24.7 | 18.4 | 20.4 |
| 25 | HDUS8 | 8.34 | 34.2 | 22.4 | 18.2 |
| 26 | HDUL1 | 7.28 | 70.2 | 68.8 | 66.8 |
| 27 | HDUL2 | 9.22 | 35.6 | 24.8 | 32.2 |
| 28 | HDUL3 | 7.42 | 84.2 | 82.4 | 78.2 |
| 29 | HDUL4 | 7.50 | 86.0 | 82.8 | 81.2 |
| 30 | HDUL5 | 7.24 | 74.6 | 72.4 | 70.4 |
| 31 | HDUL6 | 7.80 | 68.9 | 60.4 | 62.6 |

| | | | | | |
|---|---|---|---|---|---|
| 32 | HDUL7 | 9.21 | 26.8 | 21.8 | 22.4 |
| 33 | HDNS1 | 8.42 | 42.8 | 38.6 | 28.8 |
| 34 | HDNS2 | 4.56 | 42.6 | 32.2 | 24.6 |
| 35 | HDNS3 | 4.10 | 36.2 | 24.6 | 32.4 |
| 36 | HDNS4 | 3.45 | 22.3 | 18.8 | 20.6 |
| 37 | HDNS5 | 3.56 | 18.4 | 21.4 | 18.6 |
| 38 | HDNS6 | 4.82 | 26.6 | 28.4 | 28.8 |
| 39 | HDNS7 | 6.24 | 58.8 | 44.8 | 38.6 |
| 40 | HDNS8 | 7.24 | 74.6 | 72.4 | 72.4 |
| 41 | HDNS9 | 7.52 | 82.8 | 79.0 | 80.8 |
| 42 | HDNL1 | 7.40 | 84.6 | 78.8 | 78.2 |
| 43 | HDNL2 | 7.25 | 78.2 | 75.4 | 74.2 |
| 44 | HDNL3 | 7.65 | 74.2 | 68.8 | 66.2 |
| 45 | HDNL4 | 4.75 | 34.6 | 24.8 | 28.8 |
| 46 | HDNL5 | 3.84 | 22.4 | 18.6 | 22.4 |
| 47 | HDNL6 | 3.90 | 26.8 | 35.4 | 28.2 |
| 48 | HDNL7 | 4.50 | 34.2 | 24.6 | 22.8 |
| 49 | HDNL8 | 5.60 | 68.9 | 64.2 | 68.8 |
| 50 | HDNL9 | 6.52 | 75.8 | 62.4 | 68.2 |

**Biological Oxygen Demand (BOD)**

BOD3 is considered to be an important pollution parameter to examine the water quality. The presence of fats, nutrients, lactose, detergents, sanitizing agents, casein and inorganic salts in dairy wastewater results in its high BOD3 values, thus making water unfit for drinking and irrigation purposes [22], [23]. Therefore, BOD3 values of dairy wastewater should be estimated before its discharge to the environment. Only eight isolates were efficient in reducing BOD3 content above 80%. Maximum percentage BOD3 reduction was shown by isolate VDLL5 i.e. 88.5% (90 mg/l) where as a reduction in BOD3 of control was only 12.5% (900 mg/l). Isolate HDUL4, HDNL1 were also efficient in reducing BOD3 content by 86.0%, 84.6% respectively. Bioremediation of industrial wastewater using microbial isolates showed a high reduction of BOD3. The reduction in BOD3 values could be associated with the consumption of organic matter by the microbial isolates. Silambarasan et al. [24] reported that 64.67% reduction of BOD3 was observed by bioaugmenting *Pithophora sp* in dairy wastewater. Significant reduction in BOD3 values of dairy wastewater by microbial isolates has also been reported by Das and Santra [25], Gaikwad et al. [26]. According to Marwaha et al. [27] *Candida parapsilosis*, MTCC 1965 showed the reduction in BOD content of dairy wastewater by 72%.

**Total Suspended Solids (TSS)**

TSS is also one of the important pollution parameter used to evaluate the pollution potential for dairy wastewater and also to determine the efficiency of the treatment unit [1]. Suspended solids in the wastewater originate from gelatinous milk and the curd fines or flavorings [28]. Total suspended solids (TSS) of unorganized dairy sector wastewater ranged between 410–900 mg/l. The high level of total suspended solids is due to the organic and inorganic matter present in wastewater. The presence of total suspended solids in wastewater increases turbidity, reduces light penetration in receiving water bodies, and can also affect aquatic life by clogging fish gills [29], [30]. TSS in control was 450 mg/l. By bioaugmenting dairy water with bacterial isolates, TSS was reduced to 100 mg/l by four isolates. TSS reduction above 80% was shown by four isolates. Highest TSS reduction (about 82.8%) was shown by isolate HDUL4, HDUL3. Priya et al. [31] reported the percentage reduction in TSS content of dairy wastewater up to 83.4% by *Streptomyces indianesis* ACT 7 isolated from dairy wastewater. Shruthi et al. [32] had also reported a 75.7% reduction in TSS of rubber processing wastewater by using *Pseudomonas sp*. Gaikwad et al. [26] found similar results for the reduction in TSS content by 79.76% by using microbial consortia of various bacterial species, namely *Actinomycetes, Bacillus, Pseudomonas, Staphylococcus,* and *Streptomyces*.

**Oil and grease Content**

The presence of oil and grease content in wastewater forms films on the water surfaces and thus reduces oxygen transfer rates, creating high oxygen demands [31]. Oil and grease content of untreated dairy wastewater were in the range of 218–700 mg/l. Bioaugmented dairy wastewater with bacterial isolates reduces oil and grease content up to 30 mg/l. Four isolates show oil and grease content reduction above 80%. Isolate VDLL6 is more efficient in reducing the oil and grease content by 82.8%. Vida et al. [20] reported that the bacterial isolate having bacilli like characteristics were found to, be most effective in reducing the fat content of the dairy waste by 55%. According to Porwal et al. [1], the isolate DSI3 was efficient in reducing oil and grease content of dairy wastewater by 96.9%. Isolate no VDLL1, VDLL5, and HDUL 4 were selected based on their efficiency to reduce the three major pollution potential parameters, i.e., BOD, TSS and Oil and grease content. Graphic representation of bioaugmentation efficacy of the selected isolates were shown in Fig. 2.

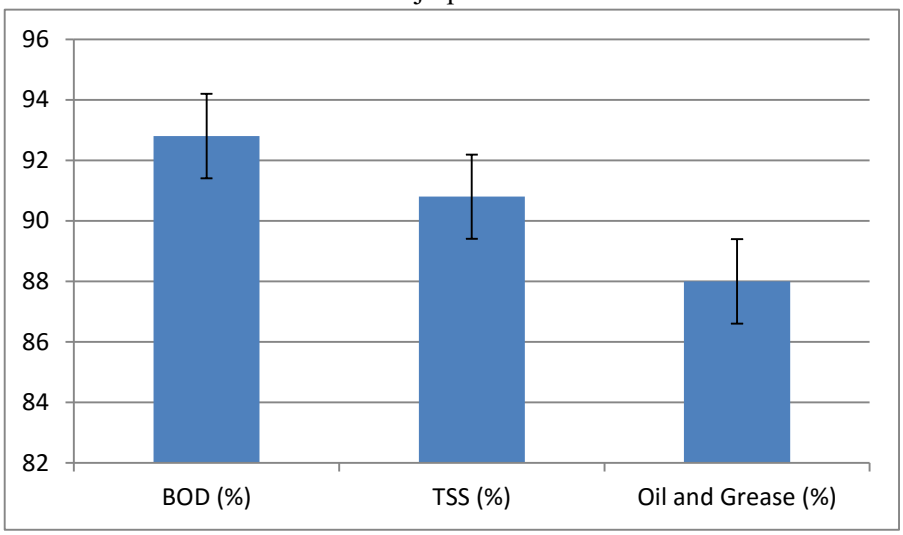


**Fig. 2: Graphic representation of bioaugmentation efficacy of the ten most effective isolates**

**Identification of Bacterial Isolates**

Extraction of DNA from the selected bacterial isolates was done as per the procedure described by Atashpaz et al. [13]. The quality and intactness of the extracted DNA was examined by running on 1% agarose gel, which contains 1 µg/ml ethidium bromide. The A260/A280 absorbance ratio of the extracted DNA samples was found to be nearer to 1.8 (Table 3). The extracted DNA molecules were used as templates for the amplification of 16 S rRNA genes. The universal primers 27 F and 1492 R were used for the amplification of 16 S rRNA genes at the annealing temperature of 55 °C. The intense single bands were observed on 1% agarose gel stained with ethidium bromide.

**Sequencing results**

For bacterial classification, generally, sequencing of 16 S rRNA gene was used as an important identification tool [33]. The reasons include its presence in almost all bacteria; its function has not changed over time, and the 16 S rRNA gene (1,500 bp) is large enough to provide a genus and species identification for isolates [34-37]. The DNA samples of all the bacterial isolates were run on the agarose gel, and the bands were visualized when observed under the Gel doc. The sequencing of the 16 S rRNA gene was done. Based on the 16 S rRNA sequences, phylogenetic dendrograms were constructed to know the genetic relationship between the bacterial isolates. The identification of the isolates was represented in Table 4, and their phylogenetic dendrograms were shown in the (Fig. 3, 4 and Fig. 5).

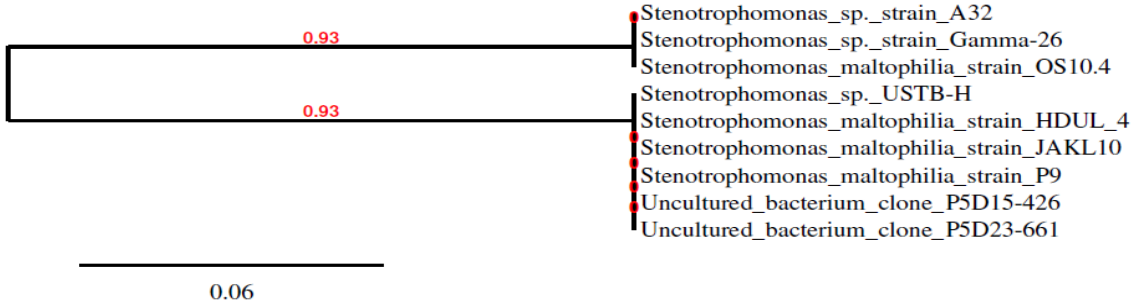


**Fig. 3: Phylogenetic tree showing close homologs to *Stenotrophomonas spp*.**

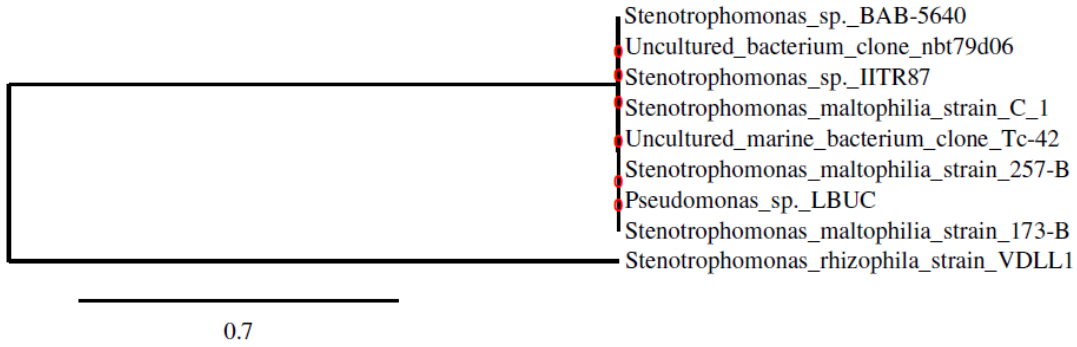


**Fig. 4: Phylogenetic tree showing close homologs to *Stenotrophomonas spp*.**

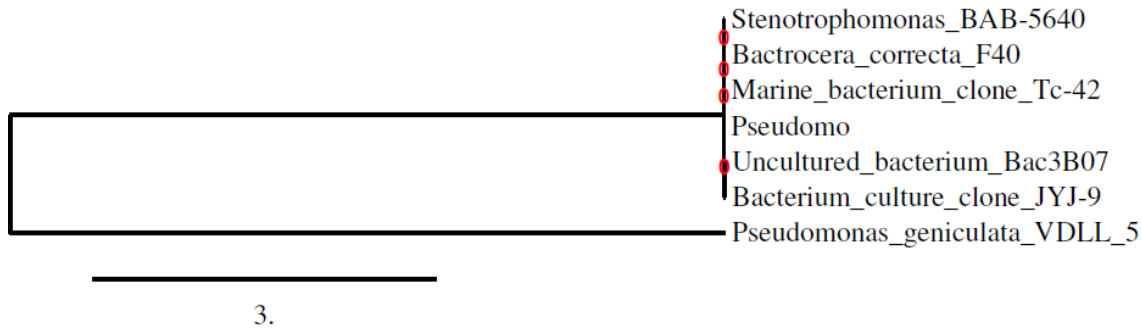


**Fig. 5: Phylogenetic tree showing close homologs to *Pseudomonas spp*.**

## 4. CONCLUSION

Environmental laws have become stringent, discharge of the effluent within the permissible limit is mandatory in the developed and developing countries. The dairy industry is practiced at a large scale as well as at a small scale level all over the world. The dairy wastewater treatment methods practiced by large-scale holders comprise physicochemical methods requiring a large surface area for the setup of effluent treatment plant and technically trained personnel with efficient management skills. It adds to the cost of the treatment process, making it cost-intensive and cannot be employed in small scale industries. Therefore, biological treatment methods are considered to be ideal and economical. Dairy industry wastewater is an enriched media for microbial growth. They do not contain hazardous materials and being organically rich; they are an ideal candidate for biological treatment which is carried out by indigenous microflora. Indigenous microflora increases the efficiency of the biological treatment system as they were adapted to the wastewater physical-chemical conditions. The present investigation was carried out to isolate the most frequently occurring and optimally performing microorganisms from dairy wastewater and sludge samples. Three bacterial isolates VDLL1, VDLL5 and HDUL 4 were selected based on their bioremediation efficiency to reduce BOD, TSS and Oil, and grease content. 16 S rRNA sequencing results concludes that all of the selected strains belong to Stenotrophomonas spp. The maximum reduction in BOD3 was shown by isolate VDLL5 i.e. 88.5%. Isolate HDUL4 and HDUL3 were efficient in reducing the TSS content by 82.8% and 82.4%. Isolate no. VDLL6 and VDLL7 were more efficient in reducing the oil and grease content by 82.8% and 82.6% respectively. As per the standards set by Central Pollution

Control Board, New Delhi for the discharge of dairy wastewater to the surface water, BOD of the treated wastewater should be not more than 100 mg/l (if applying on land), TSS content should be 150 mg/l and Oil and grease content should be 10 mg/l. Bioaugmentation of dairy wastewater by these selected isolates reduced the BOD3, TSS, and Oil and grease content up to 50 mg/l, 100 mg/l, and 40 mg/l, respectively. So, the treated dairy effluent is suitable for applying on the land for irrigation purposes. The identification of these active strains will lead to the development of suitable, eco-friendly method for the treatment of dairy wastewater. These findings are of great concern as overall efficiency of the treatment process will be increased by bio-augmenting dairy wastewater with optimally performing strains isolated from the same source as dairy wastewater exhibits dynamic characteristics, it is always better to use consortium over single culture. Currently work is underway to construct the microbial consortia based on individual efficacy of isolates.

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**CONFLICT OF INTEREST**

Authors have no conflict of Interest

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