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ASSESSMENT OF BACTERIAL CONSORTIA FOR THE EFFECTIVE BIOLOGICAL TREATMENT OF DAIRY EFFLUENT

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ABSTRACT

The dairy effluent sample was analyzed for its physicochemical characteristics. Four potential bacteria *(L2 – Lysinibacillus sphaericus, L8 - Pseudomonas taiwanensis, P11 - Bacillus marisflavi and P9 - Pseudomonas aeruginosa)* were isolated from dairy effluent and their individual efficacy was evaluated with physicochemical parameters. All the four bacteria showed appreciable degradation with P9 at higher order followed by L2. To improve the effectiveness in removal, five sets of bacterial consortia were designed by integrating the isolates in proportion with different combinations and treated with the effluent. The reports showed that consortia set C3 dominated with the maximum level of removal of BOD (88.31%), COD (79.51%), TDS (41.66%), Protein (74.44%) and Lipid (72.87%) when compared to the removal by other sets. From these results of the present study, it is evident that the consortium C3 could be employed for the biological treatment of the dairy effluent in effective manner.

KEYWORDS: ***Bacterial consortia, Biological treatment, Biodegradation and Dairy effluent***.

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INTRODUCTION

Dairy industry is found worldwide but India ranks first among the maximum major milk producing nation[1]. The average composition of milk is 87.20% water and 12.80% dry matter (fat, proteins, lactose) [2]. Dairy industry in India, on an average, generates effluent of about 6-10 L for one litre of processed milk, containing high organic and inorganic contents[3]. The increased milk demand has led to rapid growth of industries which enhanced the production and release of toxic substances into the environment[4]. The effluent and its individual components are found to be detrimental not only for human but also to various flora and fauna. The characteristics of a dairy effluent contain Temperature, Color, pH, BOD, COD, Chlorides, Sulfates, Oil and Grease. The effluent production and disposal remain a problematic issue as they affect the ecosystem[5]. The best way to deal this problem in an eco-friendly manner is via bioremediation. Bioremediation is the process of enhancing the performance of the treatment systems by adding external microorganisms with high degradation capacity[6]. Currently a wide range of microorganisms (bacteria, yeast, fungi, and algae) are being studied for use in bioremediation processes. Considering the above stated implications, a research has been conducted and is discussed in the present work to understand the bioremediation potential by means of aerobic biological treatment of individual microorganism with the effluent and its efficacy when prepared as consortium.

MATERIALS AND METHODS

The effluent was collected from the Aavin Dairy plant, Chennai in a sterilized container. The potential bacterial strains were isolated, sequenced and identified as L2 – *Lysinibacillus sphaericus*, L8 - *Pseudomonas taiwanensis,* P11 - *Bacillus marisflavi* and P9 - *Pseudomonas aeruginosa* by 16s rRNA analysis (Figure 1). To find out the optimum inoculum concentration, the dosage levels of inoculums were varied between 2-15% in the effluent. The optimum amount was then found in terms of BOD and COD (Figure 2 and 3). For determining of individual efficacy of the isolates in reducing pollutants, they were treated to the effluent sample individually with optimum inoculums dosage and the various physicochemical characteristics such as pH, Temperature, BOD, COD, TDS, Chloride, Sulfate, Protein, Lipid, Oil and Grease were analyzed as per the method described by APHA (American Public Health Association) (2012) in standard methods. Those physicochemical parameters were also analyzed for the untreated effluent sample for comparison.

Preparation of Consortium

To study the combined efficacy of the isolates, five sets of microbial consortia such as - C1 (P9, P11, L2), C2 (L8, L2, P11), C3 (L8, L2, P9), C4 (P9, P11, L8) and C5 (L8, L2, P9, P11) were designed and added in proportion to the dairy effluent[7].

Figure 1
Shows Bacterial isolates P9, P11, L2 and L8

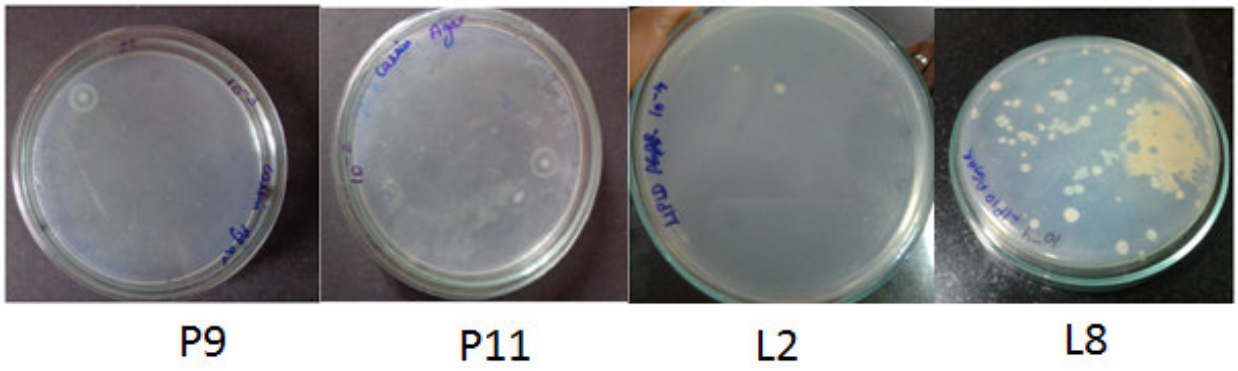


Figure 2
Shows 5% dosage level as optimal inoculum percentage for P9 and P11. Data represent mean ± S.D. (n=3); P < 0.05

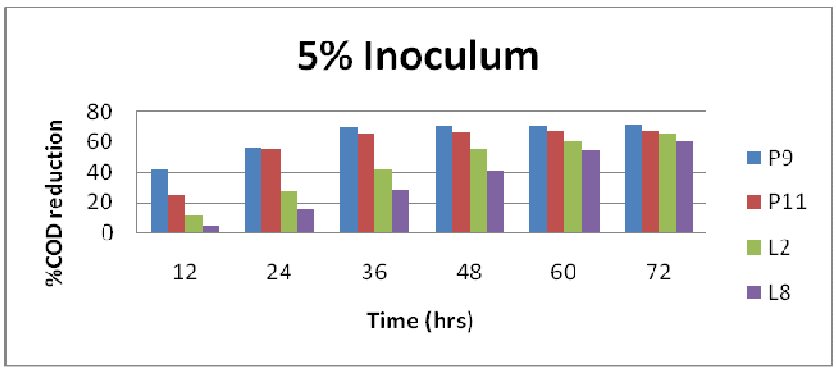
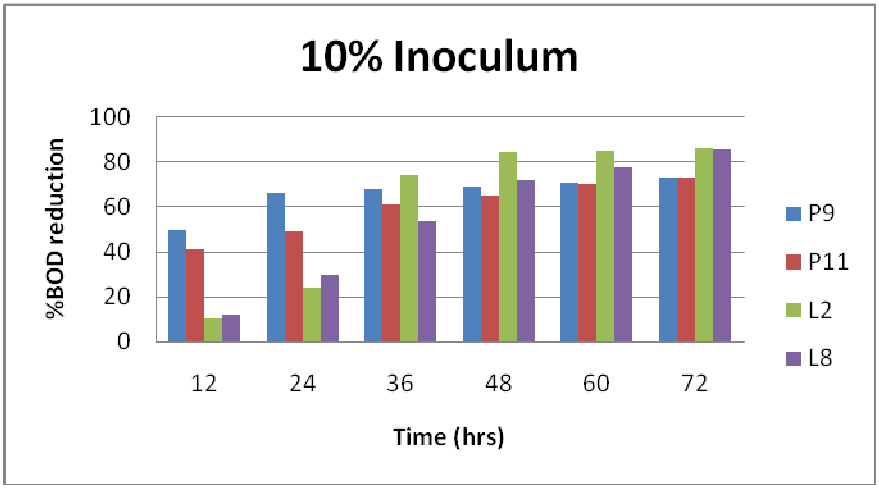


Figure 3
Shows 10% dosage level as optimal inoculum percentage for L2 and L8. Data represent mean ± S.D. (n=3); P < 0.05



RESULTS AND DISCUSSION

The bioremediation potential of microorganisms and its maximum efficacy in the effluent treatment is determined by the addition of optimal amount of inoculum. The dairy effluent was treated with varied levels of inoculum between 2-15% in order to determine the optimal amount of inoculum dosage in terms of percentage BOD and COD reduction. The obtained results showed that the inoculum level of 5% v/v of P9 & P11 with maximum reduction of BOD & COD than other level of inoculums (Figure 2). For L2 & L8, the inoculum level of 10% v/v exhibited maximum reduction of BOD and COD (Figure 3).From the previous research findings, the optimal inoculums percentage for maximum effluent removal used was 2% [8], which correlates with our study and a bit further, the removal was found more efficient with 5% for *Pseudomonas sp.* and 10% for *Bacillus sp.*

1. Analysis of physicochemical parameters of dairy effluent

To analyze the individual effectiveness of the microbial isolates, it is necessary to find the physicochemical parameters such as pH, Temperature, BOD, COD, TDS, Chloride, Sulfate, Protein, Fat, Oil and Grease. The standard limits are as follows: pH- 5.5-9, BOD-30mg/l, COD-250mg/l, TDS- 2100mg/l, Sulfate – 1000mg/l, Chloride- 600mg/l, Oil & Grease – 10mg/l.

(i) pH

The pH was determined by direct measurement with a pH meter. It was found to be acidic for the untreated sample due to decomposition of lactose under aerobic conditions. The effluent with acidic pH could have an adverse effect on soil and micro flora[9]. The effluent was treated with microbial isolates individually and it was found that pH was variable from slightly acidic to alkaline

Table 1
Physicochemical Analysis of Dairy effluent (Untreated)

Time (hrs)	pH	Temperature	BOD (mg/l)	COD (mg/l)	TDS (mg/l)	Chloride (mg/l)	Sulfate (mg/l)	Protein (mg/l)	Lipid (mg/l)	Oil & Grease (mg/l)
0	9	34	190	730	1825	603	16	1.2	8.634	4
12	9	34	190	729	1823	602	16	1.2	8.629	4
24	9	34	189	729	1832	602	16	1.24	8.628	4
36	9	34	187	735	1821	599	15	1.21	8.643	3
48	9	34	191	727	1820	611	14	1.15	8.617	3
60	8.9	34	189	732	1835	599	16	1.13	8.651	4
72	9	34	186	725	1817	604	13	1.2	8.601	3
Mean ± S.D	8.99 ± 0.005399	34	188.86 ± 0.5577	729.57 ± 1.58	1824.71 ± 2.5033	602.86 ±1.8012	15.14 ± 0.3415	1.18 ± 0.01661	8.63 ± 0.0048	3.57 ± 0.07636

(ii) Temperature

It is important to note the temperature change, as increase in temperature affects the metabolic activities of the microbial population and helps in determining the solubility of oxygen, bicarbonates and carbon dioxide. No specific limit is prescribed by WHO (World Health Organization) or PCBI (Pollution Control Board of India) for temperature[10]. The Temperature was determined by direct measurement with a thermo gun (Raytek Class II laser). In the present investigation, the temperature value for untreated effluent was 34 ° C which remained same with the treated sample.

(iii) BOD

It is widely used as an indication of the organic quality of water. It is used for the determination of amount of dissolved oxygen required to break down organic material by an aerobic biological organisms at a certain temperature over a period of time[11]. Porwal et al. suggested that the BOD reduction can result in simultaneous reaction of coliform populations[12]. In the

present study, Dilution method was used to find the amount of BOD present in the effluent. From the observed values, the value of BOD for untreated effluent was 188.86 ± 0.5577 mg/l which is beyond the allowable limits of TPCB (Tamil Nadu Pollution Control Board) (Table 1). For samples treated with the isolates, the reductions in the BOD values were found significantly over a time period. Among themselves, the maximum reduction with permissible limit of 86.32% (26 mg/l) was seen with L2 isolate in 90hrs (Table 2). Our result is in agreement with work of Noorjahan CM et al.[13] whose BOD values of untreated effluent was 700 ± 1.5811 mg/l and those when addition of isolate reduced to allowable limit of 70 mg/l with 90% reduction.

(iv) COD

This test is used to determine the amount of oxygen required for the oxidation of organic matter to produce carbon dioxide and water. Usually COD values are higher than the BOD values since many organic substances are difficult to oxidize biologically [14]. Open reflux titrimetric method was used in our study to determine the COD values. For untreated sample, the COD value was recorded as 729.57 ± 1.14504 mg/l which is beyond the permissible limit. In case of treated samples, maximum reduction of COD value of 71.36% (209 mg/l) in 90hrs within allowable limit was found with P9 isolate (Table 3). Loperena et al. has reported that *Bacillus* strains removed COD in the range of 34-57% and *Pseudomonas* strains removed COD in range of 26-31%, which is in comparable with our reports[15].

Table 2
% BOD reduction of isolates

%REMOVAL OF BOD				
Time (hrs)	**P9**	**P11**	**L2**	**L8**
0	0	0	0	0
12	52.11	43.68	10.53	12.11
24	66.84	50	24.21	29.47
36	70.53	63.68	74.21	53.68
48	72.63	67.89	84.74	72.11
60	73.68	72.11	85.26	77.89
72	78.42	76.84	86.32	85.79

(v) TDS

It is considered a secondary pollutant and as an indicator of the presence of broad array of chemical contaminants [16]. It measures the combined content of all organic and inorganic substances in the effluent. In this study, the TDS values are measured by using Gravimetric method. The TDS amount present in the untreated sample is depicted in the Table 1 as 1824.71 ± 2.5033 mg/l which is beyond the permissible limit. The reduction in TDS values was found in the treated samples and among them, the maximum reduction of 27.67% (1320 mg/l) was recorded with P11 isolate in 90hrs which lies within the permissible limit (Table 4). Shruti et al.[17] reported reduction in TDS of an effluent using *Pseudomonas Sp.* of about 68.8% which is in accordance with our findings.

Table 3
% COD reduction of isolates

% REMOVAL OF COD				
Time (hrs)	**P9**	**P11**	**L2**	**L8**
0	0	0	0	0
12	42.05	24.79	15.75	7.53
24	56.16	55.61	31.64	18.77
36	69.72	65.89	56.43	33.56
48	70.68	66.43	59.86	52.6
60	70.68	67.12	60.68	62.05
72	71.36	67.26	69.31	68.63

(vi) Chloride

It is one of the major anions which could be toxic to aquatic life at significant concentrations. The Chloride content was measured using Stannous chloride method. The values obtained for untreated sample was 602.86 ± 1.8012 mg/l which is not within the permissible limit. In treated samples, the isolate P9 showed maximum reduction of 64.84% (212 mg/l) in 90hrs, which is also within the limit. Similar kind of reduction was studied by Saranraj et al. [18] by using *Bacillus sp., Pseudomonas sp., E.coli* in a sugar mill effluent.

(vii) Sulfate

It is one of the major cation that can have laxative effect in water at high concentration. Persulfate method is followed in our study to find the values of sulfate. For untreated sample, the value of sulfate was observed as 15.14 ± 0.3415 mg/l. In treated samples, the maximum sulfate reduction of 62.5% (6 mg/l) was observed with P11 isolate in 90hrs which is within the permissible limits. The obtained values are in accordance with values reported by Murugesan et al.[19].

Table 4
% TDS reduction of isolates

% REMOVAL OF TDS				
Time (hrs)	P9	P11	L2	L8
0	0	0	0	0
12	12.33	7.39	4.49	2.95
24	18.63	17.15	11.17	6.52
36	19.78	20.6	15.78	8.87
48	20.11	21.26	98.03	13.47
60	20.93	22.02	16.27	17.09
72	22.14	24.93	17.15	18.24
90	27.18	27.67	18.24	20.87

(viii) Protein

Reduction of protein content is necessary in a dairy effluent as they cause problems by decreasing the nitrogen transfer rate at higher concentrations. It was observed in our study that untreated sample had protein content of 1.18 ± 0.01661 mg/l and in treated sample the protein content was reduced to 0.4mg/l by using P9 isolate with maximum reduction of 65.83% in 90hrs.

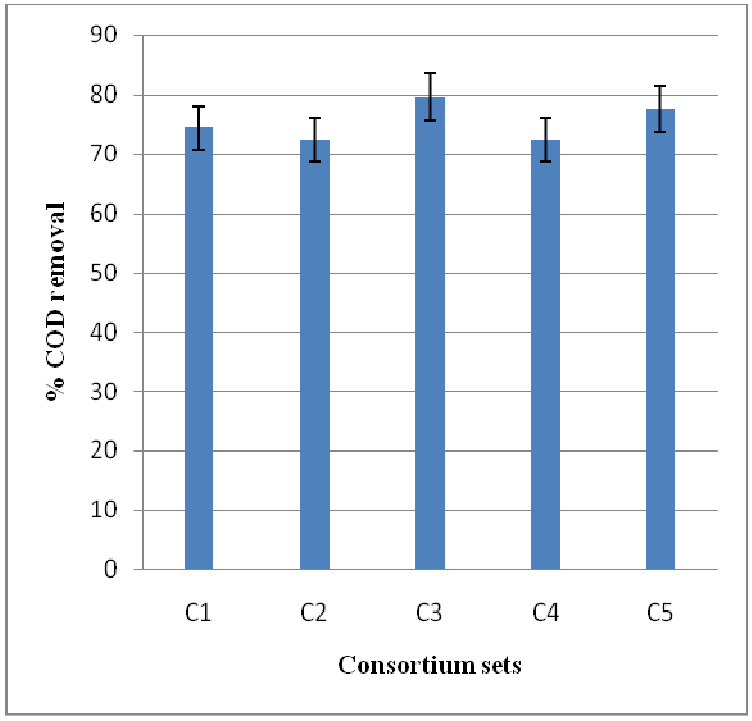
(ix) Lipid

Lipids are major organic biomolecules that cause problems at high concentrations by decreasing the oxygen transfer rate. In our study, the lipid content of untreated sample was found to be 8.63 ± 0.0048 mg/l which was further reduced in the treated sample to 2446 mg/l (71.67%) with P9 isolate in 90hrs. In parallel to our findings, Prasad et al. reported that *Pseudomonas aeruginosa* showed good potential for reduction in lipid content[20].

2. Effect of Consortium sets

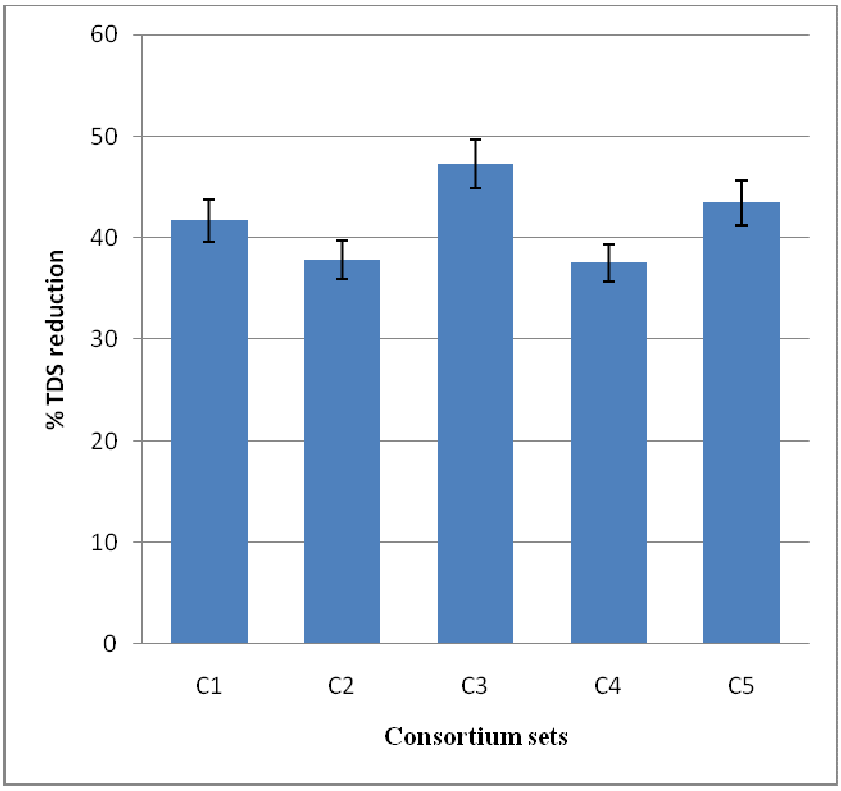
The obtained results reports that consortium C3 shows maximum reduction of BOD content of about 88.31% in 90hrs when compared with other four consortiums, which is also higher than removal percentage of BOD by individual organism. In case of COD reduction, all the five sets showed appreciable degradation with consortium C3 a slightly higher removal percentage of 79.51% in 90hrs, which is also higher than removal percentage of COD by individual organism (Figure 4).

Figure 4
Effect of consortium in percentage COD reduction.



In case of TDS, there was no higher reduction was done by the isolates. The set C1 showed reduction of 41.66%, C2 with 37.78%, C4 with 37.52% and C5 with 43.44%. The set C3 exhibited higher removal percentage of TDS of about 47.31% in 90hrs, which is also higher than removal percentage of TDS by individual organism (Figure 5).

Figure 5
Effect of consortium in percentage TDS reduction.



In case of Protein removal, the set C4 showed maximum removal efficiency of about 76.66% followed by set C3 with 74.44% in 90hrs, which is also higher than removal percentage of protein by individual organism. Appreciable amount of degradation was seen with lipid content with consortium sets, as set C3 showed higher degradation of about 88.76% followed by set C2 with 72.87% reduction in 90hrs, which is also higher than removal percentage of lipid by individual organism.

CONCLUSION

The overall study of the physicochemical parameters showed that all the individual isolates added to the effluent sample were found to be effective in reducing the organic pollutants of dairy effluent. When compared among them, the maximum efficiency was shown by *Pseudomonas aeruginosa* isolate followed by *L2 – Lysinibacillus sphaericus*. The reports obtained from the treatment of consortium sets with the effluents exhibited that, set C3 (*L2 – Lysinibacillus sphaericus, L8 - Pseudomonas taiwanensis* and *P9 - Pseudomonas aeruginosa)* dominated with the maximum level of removal of lipid, protein, COD, BOD, and TDS when compared to the removals by other sets. It also illustrated a suitable combination of the potential isolates that may be employed for efficient biological treatment of dairy effluent.

CONFLICT OF INTEREST

None of the authors has any conflicts of interest to disclose and all authors support submission to this journal.

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