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Biodegradation of dairy effluent using *Bacillus subtilis* and *Pseudomonas aeruginosa*

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ABSTRACT

Water is a major constituent in the diary industry, which results in significant effluent volumes being generated; hence the challenge of its disposal cannot be ignored. The Aavin, industy is unable to pay the full cost of managing industrial effluents, which inadvertently encourages environmental pollution. From this raw effluent, bacterial species are isolated, and the isolated species are identified. Among this bacterial isolates two dominants species *Bacillus subtilis* and *Pseudomonas aeruginosa* where selected for effluent treatment process. The two organisms were inoculated in to the dairy effluent separately and incubated for few days. After incubation the physicochemical parameters such as BOD, COD, PH, Colour, Odour, Alkalinity, Total solids, Total dissolved solids, Chlorite, and Turbidity were analyzed both in the raw and treated effluent water. From the result of the present study it is evident that *Pseudomonas aeruginosa* could be employed for effluent treatment process in large industrial level.

Key words: Dairy effluent, *Bacillus subtilis, Pseudomonas aeruginosa*, physico-chemical parameters.

INTRODUCTION

In the developing countries, rapid urbanization and industrialization is making the satisfactory collection, treatment and disposal of physico-chemical parameters a formidable problem with serious implications for public health[1].Many industrial products which have aromatic compounds contaminate the ground water and gets accumulated in plants/animals leading to serious toxicological problems. The dairy waste effluents are typically, toxic, colored, inorganic, organic, and turbid with high suspended solids. Biological waste water treatment is the most widely accepted process due to its easy of handling and economic feasibility[2]. Basically it reduces the pollutants concentration through microbial coagulation and removal of non-settable organic colloidal solids. The dairy is one of the major contributors among the food industry both in terms of value and effluent. The biggest share comes from cheese and ice cream factories[3, 4, 5]. The relatively high concentration of organic matter in the dairy effluent makes it peculiar resulting in a higher biological oxygen demand (BOD) [6]. This kind of effluent should not be allowed to mix up with the municipal waste as it will result in a shock load[7]. Due to economic reasons involved in the effluent treatment, the dairy industry is very slow in taking up the treatment aspects. With increasing social awareness about the environment, the dairy industry is forced to treat its effluents effectively and efficiently before disposing into the drainage.

MATERIAL AND METHODS

Sample collection

The dairy effluent samples were collected from Avin milk industry, Trichy. The effluent was collected in polythene containers and stored at 20°C.

Isolation and identification of bacteria

Bacteria were isolated from effluent soil, adopting serial dilution method, 10 -6 and 10 -7 dilutions were used for identification. The two major isolates obtained from the samples was identified based on morphological, cultural and biochemical characteristics. The morphological characteristics included the grams staining and motility test, where as the cultural characteristics were based on growing the cultures in nutrient agar medium. The biochemical characteristics were based on Indole test ,Methyl ret test, Voges praskauer test, Citrate test, Catalase test, Oxidase test, Urease test, and Carbohydrate fermentation test . However, the biochemical tests were conducted by the following methods, as described by Cappuccino and Sherman[8].

Preparation of Inoculam

The isolated *Bacillus subtilis* and *Pseudomonas aeruginosa* were used as an inoculam. This was prepared using specific medium such as agar for *Bacillus* and isolation medium *Pseudomonas* isolation medium. The organisms were inoculated into broth medium and incubated at 37°C for 24 hours and used for effluent degradation.

Degradation studies

10 ml of two isolates namely *Bacillus subtilis* and *Pseudomonas aeruginosa* were added to 90 ml of dairy effluent separately and the standard flask culture experiments were carried out. The flask was checked microscopically for their characteristics comparing with observed in the initial inoculams. The inoculated effluent samples were incubated after different incubation time (hrs) before analyzing the physico chemical parameters.

Analytical methods

The pH of the effluent samples was recorded at the time of sample collection .Prior to colour measurement, the sample was filtered through a 0.45µm Millipore membrane filter paper to prevent turbidity and colour measurements were carried out with a spectrophotometer. Colour is determined using a UV-Visible spectrophotometer (Mode 7800 UV\VIS) by measuring the absorbance rate at three wavelengths (436,525 and 620 nm) considering the sum of these three measurements[9, 10].

The turbidity was determined by turbidity meter (HI 93703 Microprocessor Turbidity meter).COD, BOD where the physico chemical para meters were analyzed based on the standard methods[11].

RESULTS AND DISCSSION

The present study was aimed to degrade the physico chemical parameters of the effluents using microbes isolated from dairy effluents. Many different isolates were obtained from the effluent samples, but only two major colonies were considered presently and they were identified based on morphological and biochemical characteristics and also used for degradation of effluent samples. The results are shown in Table-1. It is apparent from the results that of the two isolates *Bacillus subtilis* (G+) (strain I) and *Pseudomonas aeruginosa* (G-) (strain II) were identified, morphologically *Bacillus subtilis* is raised opaque dull, grayish white colonies and *Pseudomonas aeruginosa* is translucent, bluish green pigment diffused colonies[12].

Physicochemical characteristics

The *Bacillus subtilis* and *Pseudomonas aeruginosa* was used for effluent treatment. The nature of the effluent was turn to normal when it was degraded. The results presented in the Tables – 2, 3 and Fig-1. The pH of the dairy effluent increased (7.8 pH) but after treatment with *Bacillus subtilis* and *Pseudomonas aeruginosa* it again increased to the range of neutral (7.0 pH). The previous study was Micro flora of the effluents from a dairy factory in Tehran (Pegah Dairy Processing Plant) were isolated and screened for their ability to reduce the organic matter content and COD of the effluents. 10 bacterial strains were selected due to reduction in COD content from the 4th to 6th day of incubation at 30 °C and pH =11. Highest COD reduction were obtained by two isolates, BP3 and

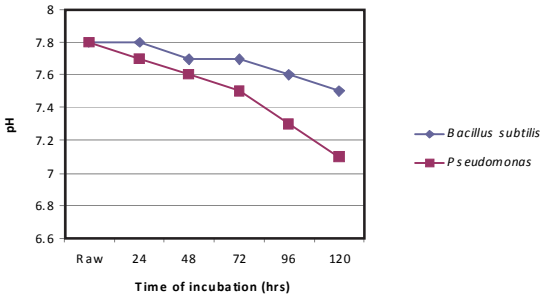

Fig. 1:

Fig

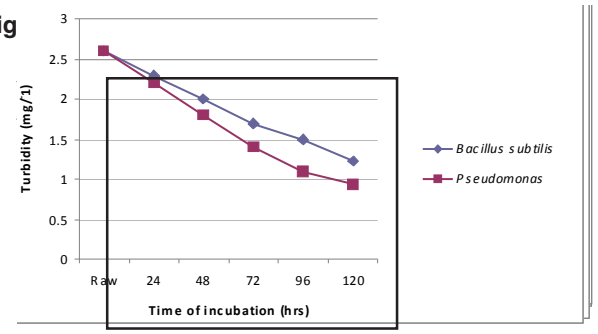

Fig. 3:

Fig. 4:

BP4, 70.7 % and 69.5 %,, respectively (The initial COD concentration was 3000 mg/l and reduced to 880 and 920 mg/l).After optimization of the condition for test organisms, big reductions in COD, carbohydrate, fat and protein content of the effluents were observed by BP3 up to 84.70 %, 98 %, 45.30 % and 53 %, respectively .The mixture of BP3 and BP4 did not show the good results as the BP3 alone .Therefore, BP3 has been selected as the most efficient microorganism for the system. The overall efficiency of the system will be increased if it is added to anaerobic activated sludge tank[13].

The initial (608mg/l) and after treatment with *Bacillus subtilis* and *Pseudomonas aeruginosa* the BOD is significantly reduced at the range of

Table 1: Identification of isolated bacterial colonies

S.No.	Properties	Strain I	Strain II
	Morphological properties		
1	Gram staining	Gram positive Rod	Gram Negative Rod
2	Motility	Motile	Motile
	Cultural Properties		
3	Nutrient agar	Raised opaque dull, white colonies,	Translucent, bluish green pigmented, diffused colonies
	Biochemical properties		
4	Indole test	-	-
5	Methyl red test	-	-
6	Voges proskauer test	+	-
7	Citrate utilization test	-	+
8	Catalase test	-	+
9	Oxidase test	+	+
10	Urease test		
11	Carbohydrate fermentation	-	-
	Glucose	+	+
	Lactose	-	-
	Mannitol	-	+

Table 2: Degradation studies of dairy effluent using *Bacillus subtilis*

S. No.	Physico chemical parameter	Raw effluent	(hrs) effluent treated with *Bacillus subtilis*				
			24	48	72	96	120
1	Colour	Yellow	Yellowish white			Colourless	
2	pH	7.8	7.8	7.7	7.7	7.6	7.5
3	Odour	Unpleasant	Unpleasant		odourless		
4	BOD (mg/l)	608	558	490	152	226	122
5	COD (mg/l)	945	845	740	652	523	42
6	$CaCo_3$ (mg/-1)	140	132	118	98	76	57
7	Total solids (mg/-1)	1960	1946	1862	1713	1564	1415
8	Total dissolved solids (mg/-1)	1350	1243	1186	1129	1072	1015
9	Chloride (mg/l)	60	50.6	41.6	32.6	23.6	14.6
10	Turbidity (mg/-1)	2.6	2.3	2	1.7	1.5	1.23

(122 mg/l) and (48 mg/l) respectively. Thus BOD is an important indication of levels of biological pollution in water and provides an index of the amount of microbiologically utilizable organic matter. Previous studies have also found decreases in coliform concentration at elevated DO concentrations and negative regression coefficients between coliform and DO concentrations[14]. In this study dairy effluent also revealed their high amount of total suspended solid (1960 mg/l). After treatment with *Bacillus subtilis* and *Pseudomonas aeruginosa* the amount was reduced to (1415 mg/l) and (1150 mg/l).

In this present study the total dissolved solids (TDS) was found to be high registering a maximum limit which may be harmful for soil characteristics in long term use for irrigation but the total dissolved solids (1350 mg/l) were significantly high in dairy effluent. It was treated with *Bacillus subtilis* and *Pseudomonas aeruginosa* these was reduced as notable amount (1015 mg/l) and (920 mg/l) respectively. Similarly studied was total dissolved solids (1360mg/L) was treated with microorganisms after treatment (<2100mg/L) reduced[15].

The Chloride concentration of the dairy effluent is high (60 mg/l) not suitable for living things. But after treatment with *Bacillus subtilis* and *Pseudomonas aeruginosa* the concentration of chloride was significantly to reduce in notable

Table 3: Degradation studies of dairy effluent using *Pseudomonas aeruginosa*

S. No.	Physico chemical parameter	Raw effluent	(hrs) effluent treated with *P. aeruginosa*				
			24	48	72	96	120
1	Colour	Yellow			Colourless		
2	pH	7.8	7.7	7.6	7.5	7.3	7.2
3	Odour	Unpleasant	Unpleasant		odourless		
4	BOD (mg/l)	608	492	360	260	144	48
5	COD (mg/l)	945	776	620	502	287	90
6	$CaCo_3$ (mg/-1)	140	188	102	86	65	38
7	Total solids (mg/-1)	1960	1794	1648	1492	1336	1150
8	Total dissolved solids (mg/-1)	1350	1124	1148	1072	996	920
9	Chloride (mg/l)	60	51	39	27	21	12
10	Turbidity (mg/-1)	2.6	2.2	1.8	1.4	1.1	0.93

amount as (14.6 mg/l and 12 mg/l) is a moderate level. The previous study was chloride concentration (104mg/l) after treatment with reduced in total chloride[16]. Similarly the dairy effluent contains high turbidity (2.6 mg/l) when after the treatment with *Bacillus subtilis* and *Pseudomonas aeruginosa* it will be decreases (1.23 mg/l and 0.93 mg/l). In this study *Pseudomonas aeroginosa* maximum level of treatment was present compared then *Bacillus subtilis.* Finally conclude that the *Pseudomonas aeroginosa* is able to isolate to use for effluent treatment process in large industrial level.

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