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Identification and study of bacteria from tannery effluent

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Abstract: *An attempt has been made to identify bacteria from secondary wastewater of existing activated sludge treatment system of effluent treatment plant. The samples were collected from effluent treatment plant at Bata India Limited, Batanagar, Mokama Ghat, Bihar, India. Few strains of bacteria viz. Enterobacter aerogens, Pseudomonas aeruginosa, Bacillus megaterium, Bacillus sp. were isolated in pure form from the effluent of activated sludge plant. The efficiency of each strain of bacteria was evaluated with reference to Biological Oxygen Demand (BOD) reduction of effluent. The bacterial strains were very active in decomposing organic matter when they act in combination. These strains were identified and their morphological tests, physiological tests, biochemical tests etc. were done. From the above study it has been found that if the effluent in activated sludge is treated with specific efficient microbes (Formulated bioculture) then better BOD reduction of effluent could be achieved.*

Key Words: *Tannery effluent, Enterobacter aerogens, Pseudomonas aeruginosa, Bacillus megaterium, Bacillus sp.*

1. INTRODUCTION :

The tanning industries, which generates huge quantities of effluent and solid wastes daily, is responsible for the pollution of environment to a great extent. Unscientific method and practices of disposal of wastes have attracted the attention of public and the tanneries are required to take all the appropriate control/management systems for abatement of pollution to meet the standards prescribed by the Pollution Control Boards.

Bioclean cultures are specifically used to resolve problems in wastewater systems receiving high-strength effluents for many industries. They have been selectively adapted through a scientific process that develops the bacteria and allows them to degrade tough and toxic compounds that would normally overwhelm naturally occurring bacteria. The Bioclean culture is designed to operate better and significantly improve conditions in problematic effluent treatment plants[1]. These microbes increase the efficiency of the plants without the need for increasing plant capacity, thereby saving electricity costs.

The present work has been taken with a view to :

- Identify the more efficient bacteria in secondary treatment process.
- Study the percentage organic load decreasing ability of the isolated pure bacteria singly and in combination.

2. MATERIALS & METHODS:

In this investigation few bacterial strains were isolated from secondary treated tannery effluent treatment plant at Bata India Limited, Batanagar, Mokama Ghat, Bihar, India during 2008.

Method of isolating pure culture:

For isolation of pure culture serial dilution and streaking methods were followed as usual in sterile agar media containing (g l^{-1}) beef extract-3, peptone-5, agar agar-15, yeast extract-3[2]. The pH of the solution was adjusted to 7 before adding agar and autoclaving.

Identification of species name of four efficient bacteria:

The identification of four pure bacterial strains and their morphological tests, physiological tests, biochemical tests etc. were conducted in the laboratory condition. These tests were carried out following the standard procedures of general characterization[2-6]. The results of the different tests carried out are shown in table 1 and table 2.

Determination efficiency of four isolated pure strains singly and in combination under atmospheric condition with respect to Biological Oxygen Demand (BOD) reduction:

The Primary treated tannery effluent samples were collected in sterilized bottle and brought back to the laboratory in an ice packed container for determination of relative efficiency of each pure strains so isolated and purified.

Effluent was disinfected with UV light in laminar flow bench. Effluent was taken in sterile BOD bottle. A small amount of a pure culture was added to the effluent. Then aeration was done with the help of sterile aerators. Initial BOD of the tannery effluent was measured following standard method. Then final BOD of effluent after aeration were also measured in the same way and maximum per cent of BOD reduction were calculated.

The BOD data was measured by using the following formula-

Fifty ml of 1% diluted primary treated tannery waste was taken to measure its dissolved oxygen content (D_1) after seeding. A centrifuge tube of 70 ml capacity is filled to the brim with the dilution water containing the sample and the seed microbe. The tube was sealed with cap and kept in the incubation chamber at 20^0 C for 5 days. The dissolved oxygen content (D.O.) was measured using an aliquot (D_2). The D.O. of the dilution water containing seed was also measured (B_1). The same was also incubated for 5 days at 20^0 C in the 70 ml centrifuge tube and then also its D.O. was measured (B_2).

$$BOD_5^{20}\ (mg/l) = \frac{(D_1 - D_2) - (B_1 - B_2) \times f}{P}$$

The value 'f' is the ratio of seed in diluted sample to the seed in seed control or the % of seed in diluted sample/percentage of seed in seed control and P = Decimal fraction of sample used[7].

The BOD reduction values by bacteria singly and in combination in atmospheric condition has been tabulated in table 3.

3. RESULTS AND DISCUSSION:

Species name and generation time of four pure bacterial strains were identified and are given in the table 1. The different characteristics of identified four species bacteria viz. *Enterobacter aerogens*, *Pseudomonas aeruginosa*, *Bacillus megaterium* and *Bacillus sp.* were done. The different tests of the said bacteria viz. morphological, physiological and biochemical tests were studied which are reflected in the table 2. The BOD reduction values by bacteria singly and in combination are given in the table 3.

Table 1. Identified bacteria from the tannery effluent

Bacteria No.	Bacteria species	Generation time of bacteria at 35^0 C temperature
1.	*Enterobacter aerogens*	30 minute
2.	*Pseudomonas aeruginosa*	36 minute
3.	*Bacillus megaterium*	35 minute
4.	*Bacillus sp.*	30 minute

Table-2. Characteristics features of isolated four pure bacterial strains through different tests:

Table –2.a. Morphological tests:

Characteristics	Bacteria			
	Enterobacter aerogens	***Pseudomonas aeruginosa***	***Bacillus megaterium***	***Bacillus sp.***
Colony Morphology: Configuration	Round	Round	Round	Round
Margin	Entire	Entire	Wavy	Wavy
Elevations	Convex	Convex	Convex	Convex
Surface	Smooth	Smooth	Rough	Rough
Density	Translucent	Translucent	Translucent	Translucent

Pigments	-	Greenish	-	-
Gram's Reaction	-	-	+	+
Shape	Rods	Rods	Rods	Rods
Size	Short	Moderate	Long	Long
Arrangement	Single	Single	Single	Single
Spore: Endospore	-	-	+	+
Position			Central	Central
Shape			Oval	Oval
Motility:	+	+	+	+
Fluorescence (UV)	-	+	-	-

Table 2.b. Physiological tests:

Characteristics	**Results**			
	Enterobacter aerogens	***Pseudomonas aeruginosa***	***Bacillus megaterium***	***Bacillus sp.***
Growth at temperatures:				
4^0C	-	-	-	-
15^0C	-	-	-	-
22^0C	+	+	+	+
26^0C	+	+	+	+
30^0C	+	+	+	+
37^0C	+	+	+	+
42^0C	+	+	+	+
55^0C	-	-	-	-
65^0C	-	-	-	-
Growth at pH:				
pH 5.0	±	±	±	+
pH 5.7	+	+	+	+
pH 6.8	+	+	+	+
pH 8.0	+	+	+	+
pH 9.0	±	±	±	±
pH 11.0	-	-	-	-
Growth on NaCl (%) :				
2.5	+	+	+	+
5.0	+	+	+	+
7.0	+	-	+	+
8.5	-	-	+	+
9.0	-	-	+	+
10.0	-	-	+	+
Growth under Anaerobic condition :	+	-	-	-

Table 2.c.i. Biochemical tests:

Tests	**Results**			
	Enterobacter aerogens	***Pseudomonas aeruginosa***	***Bacillus megaterium***	***Bacillus sp.***
Growth on MacConkey agar: non-Lacfermenter	+	+	-	-
Indole Test	-	-	-	-
Methyl Red Test	-	-	-	-
Voges Proskauer test	+	-	-	-
Citrate Utilization	+	+	+	+

Casein hydrolysis	+	+	+	+
Starch hydrolysis	-	-	+	-
Urea hydrolysis	-	-	-	-
Nitrate Reduction	+	+	-	-
Nitrite Reduction	+	-	-	-
H_2S Production	-	±	-	-
Cytochrome Oxidase test	-	+	-	+
Catalase test	+	+	+	+
Oxidation/Fermentation (O/F)	F	O	F	F
Gelatin liquifaction	-	+	+	+
Arginine dihydrolase	-	+	-	-
Lysine decarboxylase	±	-	-	-
Ornithine decarboxylase	±	-	-	-

Table 2.c.ii Biochemical tests:

Acid production from carbohydrates	**Results**			
	Enterobacter aerogens	***Pseudomonas aeruginosa***	***Bacillus megaterium***	***Bacillus sp.***
Adonitol	+	-	-	-
Arabinose	+	-	-	-
Cellobiose	+	-	+	+
Dextrose	+	+	+	+
Fructose	+	+	+	±
Galactose	±	-	+	±
Inositol	+	-	±	+
Inulin	+	-	+	+
Lactose	+	-	-	-
Maltose	+	-	+	+
Mannitol	+	+	+	+
Melibiose	±	-	-	-
Raffinose	+	-	±	-
Salicin	+	-	-	-
Sorbitol	+	-	+	±
Sucrose	+	-	+	+
Trehalose	+	+	+	+
Xylose	+	+	+	-

It is explained that single bacterial strain can reduce BOD level to a lower value, but in combination of strains more reduction of the BOD near about 100% is occurred which really means better removal of the organic compounds from the waste water (Table 3). In maximum cases, the combination of four bacteria recorded highest reduction of the BOD whereas, bacteria No. 4 showed lowest reduction of the BOD when they are in single (Fig. 1.).

Table 3. Percentage of Biological Oxygen Demand (BOD) reduction with respect to aeration time in atmospheric condition:

Bacteria No.	**6 hrs aeration**	**24 hrs aeration**	**48 hrs aeration**	**72 hrs aeration**	**96 hrs aeration**	**120 hrs aeration**	**240 hrs aeration**	**Ultimate (480 hrs) aeration**
1+2+3+4	28	75	90	98	98	98	98	98
1+2	18	64	82	90	97	98	98	98
2+3	20	57	80	90	98	98	98	98
1+4	21	60	82	92	96	98	98	98
1+3	22	58	84	90	97	98	98	98
2+4	20	55	80	91	97	97	97	97

4+3	20	56	76	86	95	97	97	97
1	18	53	68	74	88	89	89	89
2	20	50	65	76	85	86	87	87
3	20	52	60	72	86	86	86	86
4	16	50	62	70	83	84	84	84

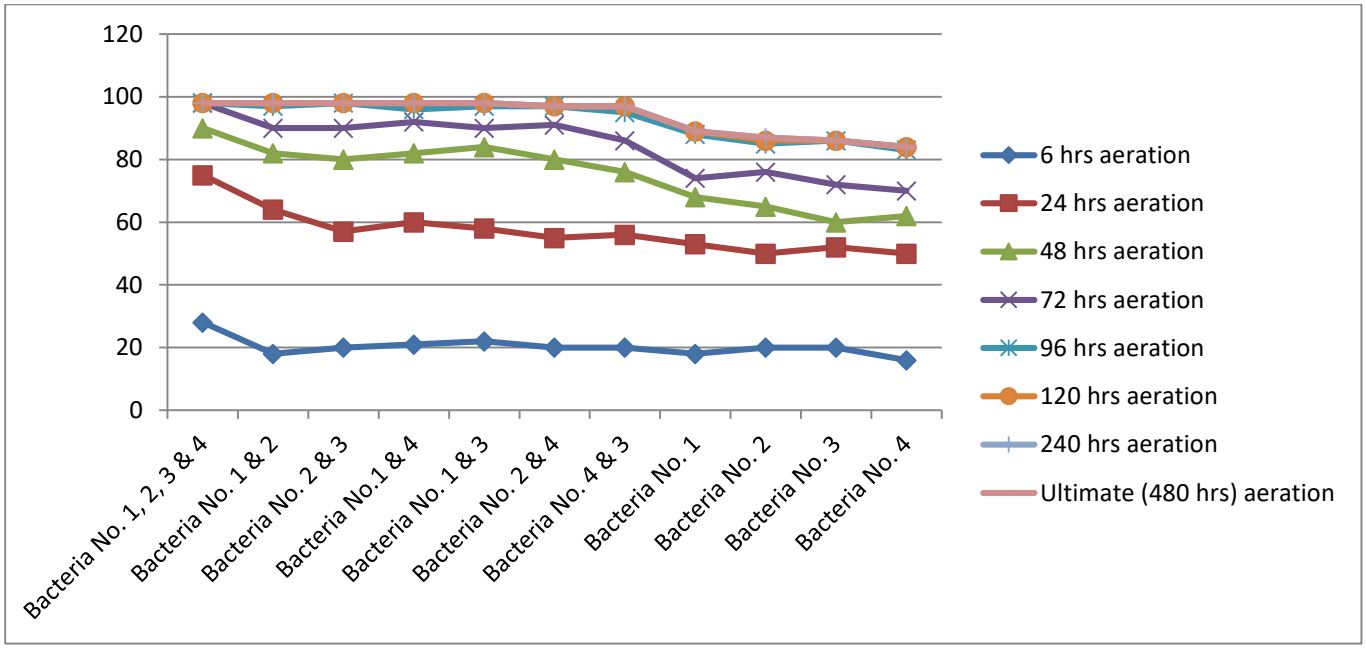

Fig. 1. Graphical presentation of percentage of Biological Oxygen Demand (BOD) reduction with respect to aeration time in atmospheric condition

4. CONCLUSION:

It will be interesting to study different types of microbes specially bacteria present in activated sludge process and formulate the bio-cultures for utilization in effluent treatment plant for reduction of organic load from tannery effluent to make the leather industry eco-friendly. No doubt the magnitude of the problem is colossal.

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