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STUDIES OF EFFICIENCY OF IMMOBILIZEDBACTERIA IN TANNERY EFFLUENT TREATMENT

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ABSTRACT

The primary purpose of wastewater treatment is to remove the suspended and soluble organic components. Biological treatment processes are used to degrade the organics (COD) in the wastewater before it is discharged. Microorganisms have a number of vital functions to control the pollution. This study aimed at, to study the efficiency of immobilized bacteria in tannery effluent treatment. In order to study the bacterial diversity in effluent and treatment of effluent by using immobilized bacteria, the effluent sample was collected from tannery industry. From this totally 6 species of bacteria such as *Pseudomonas putida, P. fluorescens, Klebsiellapneumoniae, Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis* were isolated. Among the genus Pseudomonas was found dominant group of bacteria. A great deal of effort has been made during the last three decade to establish methods to eliminate the $_{N2}$ present in the waste water. The present investigation was carried out by using immobilized bacteria to remove the pollutants from tannery waste water. Really a great result was come out from my studies .Nearly 50% successful removal of nitrogen components was observed, Chloride is difficult to remove by conventional biological treatment, in the present investigation total 24%removal was observe. Due to the immobilized bacteria the BOD and COD level was reduced nearly 75% and 65% respectively. From the present investigation given great results was observed the immobilized bacteria could be used to treat various industrial effluents.

Keywords: *isolation, identification, immobilization*

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INTRODUCTION

Water used by people and disposed into a receiving water body with altered physical and chemical parameters is defined as wastewater. If the water, however, has been contaminated with soluble or insoluble organic or inorganic material, a combination of mechanical, chemical, and/or biological purification procedures may be required to protect the environment from periodic or permanent pollution or damage. For this reason, legislation in industrialized and in many developing countries has reinforced environmental laws that regulate the maximum allowed residual concentrations of carbon, nitrogen, and phosphorous compounds in purified wastewater, before it is disposed into a river or into any other receiving water body However, enforcement of these laws is not always very strict. Enforcement seems to be related to the economy of the country and thus differs significantly between wealthy industrialized and poor developing countries.

Bioremediation technology to treat hazardous waste has gained considerable attention as it is ecologically sound and economical relative to other technologies and it has been used successfully in many countries of the world (Ritmann et al., 1988; Enrica, 1994) Microorganisms have a number of vital functions in pollution control. It is the microbial component of aquatic ecosystems that provides the self-purification capacity of natural waters in which microorganisms respond to organic pollution by increased growth and metabolism. The primary purpose of wastewater treatment is to remove the suspended and soluble organic constituents measured as chemical oxygen demand (COD).

Biological treatment processes are used to degrade the organics (COD) in the wastewater before it is discharged. The activated sludge process is the most widely applied biological wastewater treatment process in the world. In the wastewater industry uncertainty exists regarding the bacteria involved in de nitrification as well as the extent to which these bacteria contribute to nitrate and nitrite reduction under anoxic conditions. It is generally presumed that Pseudomonas spp., as well as being involved in EBPR (Osborn et al., 1989; Kavanaugh and Randall, 1994; Jorgensen and Pauli, 1995), are the predominant microorganisms through which de nitrification is achieved (Janda et al., 1988; Gray, 1990; Lazarova et al., 1992). According to Otlanabo (1993) various species of Achromobacter, Agrobacterium, Alcaligenes, Bacillus, Chromobacterium, Flavobacterium, Hyphomicrobium, Pseudomonas, Vibrio and others are responsible for de nitrification in soil. It therefore seems unlikely that only Pseudomonas spp. are responsible for de nitrification occurring in such an incredibly diverse microbial consortia as that of activated sludge. De nitrification is generally accepted as being the reduction of nitrates, via nitrites, to nitrous oxides and nitrogen gas.

However, many denitrifying bacteria only have the enzyme ability to reduce nitrates to nitrites with no further reduction of the nitrites produced (Rheinheimer, 1985; Ketchum, 1988; Cappuccino and Sherman, 1992; Robertson and Kuenen, 1992; Glass et al., 1997). In fact, according to Rheinheimer (1985) and Robertson and Kuenen (1992), most of the denitrifying bacteria in aquatic systems are only capable of incomplete de nitrification. A more intensive understa-nding of denitrifying heterotrophic bacteria is therefore essential as this may be one of the next steps to optimizing efficiency of nitrogen and phosphorus removal in nutrient removal activated sludge processes. By keeping all these in mind, the present study is aimed at,to isolate the bacteria from tannery effluent, and to identify the isolated bacteria from various biochemical test and To know the efficiency of immobilized bacteria in effluent treatment.

MATERIALS AND METHODS

Effluent Source

For the present study the effluent sample were collected from NRM Tannery industry, Trichy, Tamilnadu, India. Sample were collected in large bottles and done the Physico-chemical characteristics on the same day. Initially the pH was recorded at the collection site with BDH indicator paper. At laboratory the PH was checked again recorded at the collection meter .By using of spectrophotometer and titration methods estimated the organic and inorganic supplements (alkalinity, free co2 ,Dissolved o2, nitrate,nitrite,NH3 ,total phosphate both organic inorganic,Ca,Mg,Cl2 ,BOD and COD) initially recorded and tabulated as in Table-1.

Isolation of Bacteria from effluent

Bacteria was isolated from the effluent sample by serial dilution method and inoculated on the Agar medium for it culture and finally done the identification test by grams staining method whether the Bacterium are gram positive or gram negative.

Biochemical tests

The following Biochemical tests were done such as Mae Conkey agar test, Iodole test, Methyl red test, VogesProskauer test, Citrate utilization test, Starch hydrolysis test, Urea hydrolysis test, Nitrate reduction test, Hydrogen sulfide production test, Cytochrome oxidase test, and finally done the Catalase test for the identification of Bacteria.(Somasegaran and Hoben (1985, Josey et al 1979 and Cappuccino and Sherman, 1999).

Immobilization of Bacteria

For the immobilization of Bacteria sodium alginate was used as the immobilizing agent for bed preparation. Exponentially growing cells were harvested by centrifugation (5000xg for 10min) and re suspended in sterile water, to this alginate solution was mixed thoroughly to prepare the alginate-bacterial mixture. These alginate-bacteria mixture was then added drop wise in to Cacl2 solution for the immobilization. Thee beads were kept in the same solution for 30min at 40 c for hardening.

Experimental treatments

Experimental treatment was employed of effluents sample inoculated with immobilized bacteria (treatment) and effluents without inoculation(control). The experiment was conducted in batch cultures in duplicates for a total period of 15 days in 250ml Erlenmeyer flasks. Effluent samples (control and treated) were periodically (every five days) analyzed for various physico-chemical parameters and recorded.

Physico-chemical analysis of effluent

Various physic-chemical parameters were initially analyzed and recorded such as determination of pH by BDH indicator paper, Estimation of alkalinity, Free Carbon dioxide, Dissolved oxygen, Hardness of water ,Ca ,Mg, Cl, COD by titration methods. BOD by wrinklers method, Quantitative estimation of total nitrogen both organic and inorganic and by spectrophotometer total phosphorus was estimated.(APHA1991).

RESULTS

Isolation of Bacterial from effluent

From the present investigation total six species of Bacterial flora were isolated such as Pseudomonas putida, P. fluorescens, Klebsiellapneumoniae, Escherichia coli, Staphylococcus aureus and Bacillus subtilis were identified from the effluent sample. Among this genus Pseudomonas dominated with two species. The isolated six species of bacteria are shown in Fig.1.

Biochemical tests

Biochemical characteristics of isolated bacteria were shown in Table.1. Among the six species of Bacteria three species of Pseudomonas putida, P. fluorescens, Klebsiella pneumonia given a positive test of Mae Conkey agar test, only *Escherichia coli*, and *Bacillus subtilis* given the positive test of Iodole test, except *Klebsiella pneumonia* all species shows positive methyl red test, *Escherichia coli*, and *Bacillus subtilis* given a negative VogesProskauertest,all six species of Bacterial flora given a negative Citrate utilization test, except Escherichia coli, and *Bacillus subtilis* given a negative Starch hydrolysis test, only *Klebsiella pneumonia* given positive Urea hydrolysis test, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* given the positive Nitrate reduction test, *Pseudomonas putida*, *P. fluorescens*, *Staphylococcus aureus* given the positive Hydrogen sulfide production test, except *Staphylococcus aureus* all species given positive Cytochrome oxidase test, ,except *Escherichia coli*, and *Bacillus subtilis* reaming species given negative Catalase test

Physico-chemical analysis of effluent

pH: The pH was recorded in the effluent initially 6.8 and it was brought down to 6.7 in treated effluent (Table. 2).

Free CO2: The initial free CO2 was recorded 31 mg l -1 and it was reduced slightly from 5th day onwards. The maximum reduction was observed in 15th day (18 mg l-1) in treated effluent (Table.2).

Alkalinity: The alkalinity was recorded 108 mg l-1 initially. It was brought down from 5th onwards. The maximum (40%) percentage reduction was observed in 15th day in treated effluent (Table.2).

DO: The dissolved oxygen level was

increased in control and treated effluent. The DO level was increase 1.34 to 1.75 mg l-1 on 15th day (Table.2) in immobilized bacteria inoculated effluent.

Nitrate, Nitrite and Ammonia: The Nitrate, Nitrite and Ammonia were recorded initially 90, 48 and 42 mg l-1 respectively (Table. 2). On 15th day it was reduced nearly 50% when compared to control.

Total, inorganic and organic phosphate: Total inorganic and organic phosphate levels observed and recorded in both control and treated effluent. All the forms of phosphate were reduced from 5th day onwards (Table.2). The maximum amount of reduction was observed in 15th day when compared to control.

Calcium: The calcium level was recorded initially 83 mg l-1. It was reduced to 42 mg l-1 on 15th day (Table.2). The percentage reduction was nearly 50% than control.

Magnesium: The similar trend was noticed in magnesium level as in calcium. The 48% reduction was observed on 15th day when compared to control (Table.2).

Chloride: Chloride level was observed in 57 mg l-1 initially (Table.2) and it was slightly decreased 5th day onwards. When compared to other parameters the chloride level was reduced very less amount.

BOD and COD: The BOD and COD levels were recorded 340 mg l-1 and 610 mg l-1 initially It was reduced to 90 mg l-1 and 220 mg l-1 respectively on 15th day (Table.2).

DISCUSSION

This study of efficiency of immobilized Bacteria in Tannery effluent treatment total six species was isolated among the genus Pseudomonas was found dominant group of bacteria. It is confirmed and supported by earlier finding of Ramlake and Bhattachrjer (1992). They also suggested the polluted habitats found mostly Pseudomonas because it having ability to degrade various pollutants from water samples. The present investigation was carried out by using immobilized bacteria. For immobilization sodium alginate was used. Ramesh and Singh (1993) reported the immobilized bacteria having more efficiency to remove the suspended particles than free cells. For the evaluation of the pollution load of industrial or domestic wastewaters, a measure of oxygen requirement of pollution matter has been developed as standard parameters, which is known as Biochemical Oxygen Demand (BOD). For the present investigation the BOD level was recorded 340 mg/l initially. Before discharging any industrial effluent should be removed the BOD because it adversely affect the aquatic organisms (Gurjar, 1994).

In this present study the BOD level was reduced on 15th day nearly 75% it is inconformity the previous reports of Gurjar (1994). He reported the bacterial culture removed BOD almost completely on two weeks from the date of inoculation in the water samples. From the present study a successful removal of nitrate, nitrite and ammonia were observed nearly 50%. It is supported by earlier report of Madhu and

Pillai (1994). They reported nearly 60% of removal of nitrate and ammonical nitrogen from the fertilizer industry wastewater by using bacterium Pseudomonas sp. The total phosphorus and inorganic and organic phosphate levels were reduced from 5th day onwards. The similar study was done by various workers (Sekaran and Mariappan, 1994; Banu et al., 2001). Banu et al, (2001) reported the efficiency of removal of phosphates was more in the immobilized condition than free cells of bacteria. It supported the present investigation. Chlorides are generally considered to be one of major pollutant in effluents, which are difficult to be removed by conventional biological methods. In the present study 24% removal of chloride from the effluent was observed due to the inoculation of bacteria. In the present investigation, Most of the parameters observed in reduced level when compare to control. It is supported by previous findings.

CONCLUSION

From the present investigation totally 6 species of bacteria were isolated from the effluent. Among the isolated bacteria Pseudomonas was the dominant in the effluent. Except dissolved oxygen, all other parameters showed decreased level when compare to control. The BOD and COD level were reduced nearly 75% and 65% respectively on 15th day. From the above observation it is conclude that the immobilized bacterium Pseudomonas putida could be used to treat various industrial effluents.

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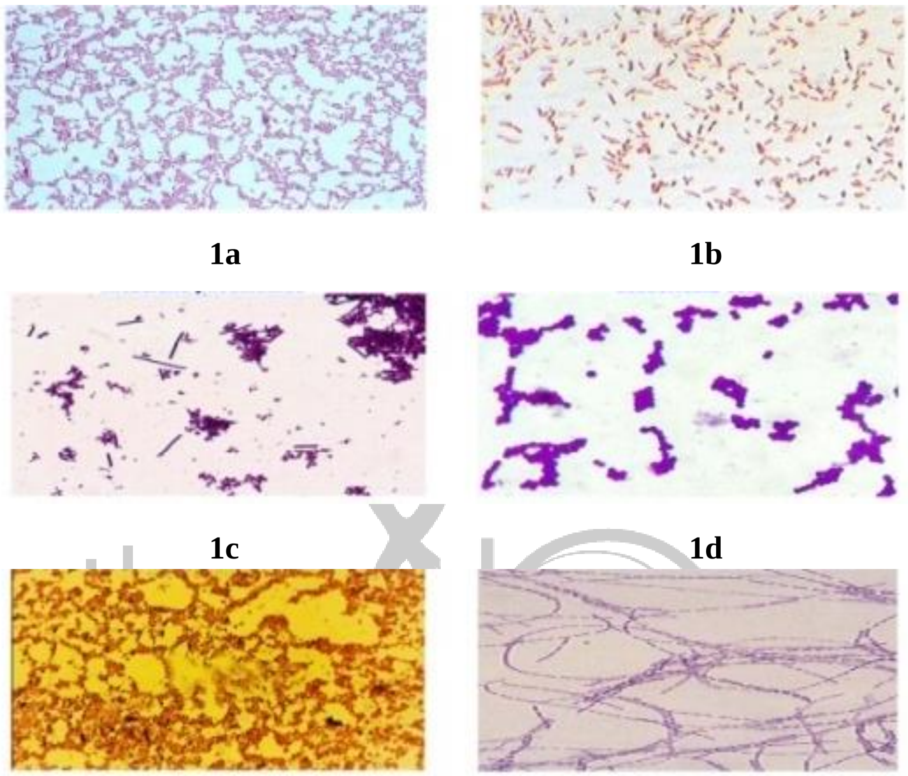
Fig-1:*Microscopic view of identified bacteria*

(1a: Pseudomonas putida, 1b: P. fluorescens, 1c: Klebsiella pneumonia, 1d: Escherichia coli, 1e: Staphylococcus aureus , 1f: Bacillus subtilis)



1a **1b**

1c **1d**

1e **1f**

Table 1: *Biochemical characteristics of isolated bacteria*

S. No	Biochemical Test	*P. putida*	*P. fluorescens*	*K. pneumoniae*	*E. coli*	*S. aureus*	*B. subtilis*
1	Mae Conkey agar test	+	+	+	-	-	-
2	Indole test	-	-	-	+	-	+
3	Methyl red test	+	+	-	+	+	+
4	VogesProskauer test	+	+	+	-	+	-
5	Citrate utilization test	-	-	-	-	-	-
6	Starch hydrolysis test	+	+	+	-	+	-
7	Urea hydrolysis test	-	-	+	-	-	-
8	Nitrate reduction test	-	-	-	+	+	+
9	Hydrogen sulfide production test	+	+	-	-	+	-
10	Cytochrome oxidase test	+	+	+	+	-	+
11	Catalase test	-	-	-	+	-	+

Table 2: *Physicochemical characteristics of effluent*

S.No	Parameters		5rd Day		10th Day		15th Day	
		Initial	Control	Treated	Control	Treated	Control	Treated
1	pH	6.8	6.7	6.7	6.7	6.7	6.7	6.7
2	Free CO_2	31	31	17	31	11	30	6
3	Total Alkalinity	108	108	75	107	45	107	18
4	DO	1.34	1.34	1.4	1.34	1.45	1.34	1.75
5	Nitrate	90	90	72	88	42	88	46
6	Nitrite	48	48	31	47	27	46	21
7	Ammonia	42	42	32	40	27	40	22
8	Total phosphate	120	116	95	114	86	114	64
9	Inorganic	65	55	46	54	38	54	32
10	Organic	55	51	40	50	29	50	21
11	Calcium	83	79	62	76	54	76	42
12	Magnesium	64	62	47	62	30	61	24
13	Chloride	57	54	49	53	44	51	41
14	BOD	340	340	260	340	220	340	90
15	COD	610	610	480	610	390	610	220

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