International Research Journal of Natural and Applied Sciences
Vol. 3, Issue 8, August 2016 IF- 3.827 ISSN: (2349-4077)
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A COMPARATIVE STUDY ON THE SYNTHETIC DYE EFFLUENT DEGRADATION POTENTIAL OF COMMERCIAL STRAIN *PSEUDOMONAS AERUGINOSA* WITH WILD SPECIES OF *PSEUDOMONAS*

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ABSTRACT

In the present study, the samples were collected from the outlet of textile mill at chinnalapatti and the physio chemical parameters such as temperature, pH, hardness, calcium, sodium, potassium, total dissolved solids, total suspended solids, chloride, electrical conductivity, dissolved oxygen and biological oxygen demand were studied. The Pseudomonas sp. was isolated by spread plate technique using King's B medium and they were indentified using various biochemical tests such as Gram's staining, motility test, and the biochemical tests such as indole, methyl red, Voges Proskauer, citrate utilization, catalase test, starch hydrolysis, gelatin hydrolysis, and casein hydrolysis. The commercial Pseudomonas aeruginosa strain was bought from Bose laboratory at Madurai. Comparative study was carried out to analyse the efficiency of the commercial strain Pseudomonas aeruginosa and the wild strain Pseudomonas sp. in different concentration such as 0.5 ml and 1 ml, and conditions such as sterile and unsterile and the percentage of decolurisation was calculated. Comparative study showed that commercial strain Pseudomonas aeruginosa was effectively degraded textile mill effluent (63%) in sterile condition at 1 ml concentration so this commercial strain could be genetically modified and used for further decolurisation of dye effluent.

Introduction

Water is the universal solvent, without life is impossible. 70% of the earth surface is covered with water. Water serves most of the human purposes such as drinking, cooking, bathing, washing, industrial and agricultural purposes. Water pollution occurs through industrial discharges. This is mainly in the form of effluent or waste water. Industries use large amount of water in their processes include, chemical manufacturers, textile manufactures, agro-industries, pulp and paper industries, metal processors etc. Among all industrial effluents, textile mill effluents contain a variety of polluting substances including dyes. Dye colour removal from spent textile dye mill is one of the consistent and persistent problems in the fields of environment. Over 7x105 metric tons of synthetic dyes are produced worldwide every year for dyeing and printing. A very small amount of dye in water (10-50mg/l) is highly visible and affects the aesthetic merit, water transparency and gas solubility of water bodies. Non judicial use of dyes in the textile industries and leather industries contribute much to the water and soil pollution, which in turn affect humans, animals and plants. The textile process requires large volume of fresh water of fairly high purity and discharge equally large volume of waste water after clothing process operations. The waste water generated contains dyes at concentration of 10-200mg/L and 10-20% lost in this effluent along with other organic materials such as fats, waxes, pectin, solid fragments, starch, derived from the cloth during its processing and constitute major source of pollution. Dyes include a broad spectrum of different chemical structures, primarily based on substituted aromatic and heterocyclic groups (Vyas and Monitories 1995). Aromatic amines are suspected as carcinogen, phenyl (C6H5-CH2) and naphthyl (NO2-OH) are ability to absorb light in the visible region. The removal o f colour from waste water is often more important than the removal of the soluble colorless organic substances, which usually contribute the major fraction of the biological oxygen demand (BOD). Colour is the first indicator to be recognized in waste water and has to be removed before discharging into water bodies or on land. Colour present in dye effluent gives a straight forward indication of water being polluted and discharge of this highly coloured effluent can damage directly the receiving water (Chen et al., 2003). Physical, physicochemical, chemical and biological processes have been investigated extensively for treating dye bearing effluents. Numerous physical and chemical techniques such as flocculation combined with flotation, electro-flotation, flocculation with Fe(II), Ca(OH2), membrane filtration, precipitation, ion-exchange, ozonation, and katox

treatment method involving the usage of activated carbon and air mixtures were also be used. Even though some of the above mentioned are effective most of them suffer from the short coming such as excess usage of chemicals, sludge disposal, expensive operating cost, ineffective colour reduction (Venkata Mohan et al., 1999). While the biological methods are inexpensive, as well as eco-friendly process. Treatment of the effluent by microbial degradation is an innovative method. It convert or degrade the pollutants into water carbon-di-oxide and various salts of inorganic nature (Low and Lee 1997; Sanche et al., 1999). The isolation of potent microbial species and degradation is one of the interest in biological aspect of effluents treatment (Bant et al ., 1996 and Aksu et al., 1997). The effectiveness of microbial decolorization depends on the adaptability and activity of selected microorganisms. The contaminated water contains heterotropic bacteria such as *Bacillus* sp. (23.3%), *Micrococcus* sp. (13.3%) *Pseudomonas* sp. (30.0%) and *Achromobacter* sp. (13.3%), Actinomycetes sp. (20.0%). (Aiso and Simudu 1962 and Gilmour et al,. 1976). In the above mentioned strains *pseudomonas* sp. is considered as on effective one in dye decolourisation. So that the *Pseudomonas* sp. are mostly used for dye degration.

Material s and Methods

Collection of sample:

The sample was collected from the outlet of textile mill at chinnalapatti, Dindigul district Tamilnadu, India. Samples were collected in a sterile container and taken to laboratory aseptically. Macroscopic features were observed and recorded. Colour and consisntency of the sample was also noted.

Analysis of physio-chemical characteristics of textile effluent:

The physio-chemical characteristics of the textile effluents such as temperature, pH, percentage of transmission, electrical conductivity, hardness, Calcium, sodium, chloride, total solids, total dissolved solids, suspended solids, dissolved oxygen, biological oxygen demand(BOD) were examined by the standard methods.

Isolation of *Pseudomonas* sp. from the contaminated soil:

King's B medium was prepared and sterilized by autoclaving at 15lbs at 121^0C for 15 minutes. Sterilized media was poured into sterile pertiplates and allowed to solidify. The textile effluent contaminated soil sample was serially diluted with sterile water blanks. Dilutions were made upto 10^-7 for bacterial emumeration, 0.1 ml from the dilutions of 10-5, 10-6 and 10-7 were spread plated on King's B agar plate and incubated at 37^0c for 24 hours.

Pure culture preparation:

Isolated cultures were streaked on separate King's B medium. Purified cultures were streaked on King's B medium slants incubated at 37^0C for 24 hours. Then the pure culture slants were stored in refrigerator.

Characterization of the bacterial isolates:

Gram's staining, Motility by hanging drop method, Biochemical tests such as indole test, methyl red and Voges-Proskauer test, citrate utilization test, starch hydrolysis, csein hydrolysis, gelation hydrolysis, utilization of glucose, catalase test were done do characterize the bacterial isolate.

Microbial activity on textile mill effluent:

Determination of dye decolorisation by *Pseudomonas* sp.:

Fourteen bottles were sterilized in hot air oven and taken out. 99ml of sample was taken in six bottles. And 99.5ml of sample was taken in another six bottles, 2 bottles were maintained as control, one for sterlilized condition another for unsterlised condition. Cultures were added in the concentration of 0.5ml and 1 ml. After inoculation the bottles were incubated at 37^0C for 5 days and then the contents in each bottle was centrifuged to determine the optical density(OD) of the sample at every 24 hours. This procedure was carried out repeatedly for five days the OD values were observed and recorded. According to the OD values the percentage of decolorisation was calculated and the graph was plotted to determine the efficiency of the cultures to

decolourise the dye effluent. Decolorisation activity was expressed in terms of percentage of decolourisation. This was calculated using the formula as described by Sani and Banarjee(1999).

$$\text{Percentage of decolorisation} = \frac{\text{Initial absorbance value} - \text{Final absorbance value}}{\text{Initial absorbance value.}} \times 100$$

Result and discussion

Macroscopic characteristics of textile mill effluent:

Macroscopic characteristics of textile mill effluent were observed and recorded. Colour and constistency of the samples were also noted. It is shown in (Table 1).

Table 1: macroscopic characteristics of tetile mill effluent:

S.No	**Characters**	**Sample**
1	Colour	Golden yellow
2	Consistency	Liquid
3	Particulate nature	Absence of particles

Analysis of physiochemical parameters of the textile mill effluent:

Physiochemical parameters such as temperature, pH, hardness, calcium, sodium, potassium, total dissolved solids, total suspended solids, chloride, electrical conductivity, dissolved oxygen, and biological oxygen demand were analyzed textile mill effluent. The results are given in (Table 2).

Table 2: Analysis of physiochemical parameters of the textile mill effluent:

S.No	Physiochemical parameters of textile mill effluent	Result
1	Temperature ^{0}C	25^{0}C
2	pH	8
3	Color	Golden yellow
4	Hardness mg/L	18800mg/L
5	Chloride mg/L	1773mg/L
6	Calcium mg/L	11mg/L
7	Sodium mg/L	13.5 mg/L
8	Potassium mg/L	20
9	Total solids mg/L	5170 mg/L
10	Total dissolved solids mg/L	3630 mg/L
11	Total suspended solids mg/L	1540
12	Biological oxygen demand [BOD] [mg/L]	38.8 mg/L
13	Dissolved oxygen mg/L	64.4 mg/L
14	Electrical conductivity mg/L	0.08 mg/L

Isolation of microorganism from the textile mill effluent contaminated soil:

Colony morphology:

Microorganism was isolated from the sample, and the colony morphology is shown in Table 3.

Table: 3 colony morphology:

S.No	Organism	Colony morphology
1.	*Pseudomonas* sp.	Large, opaque, irregular colony was observed.

Indentification of *pseudomonas* sp.:

Different tests were carried out to identify the isolated bacteria such as, Gram's staining, motility test, and the biochemical tests such as indole, methyl red, voges proskauer, citrate utilization, catalase test, starch, hydrolysis, gelatin hydrolysis, casein hydrolysis and these results are given in Table 4.

Table 4: indentification of pseudomonas spp:

Oraganism	Gram staning	Motility test	Indole test	MR test	Citrate utilization	Gelation hydrolysis	Starch hydrolysis	Catalase test	Utilisation of glucose	Casein hydrolysis	VP
Pseudomons sp.	-ve rods	-ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve

(+ve)- positive

(-ve)- negative

Determination of dye decolourization:

Dye decolurization was made by the wild and commercial strains, of pseudomonas spp under different concentration such as 0.5ml and 1ml and conditions such as sterile and unsteriled, were observed and also optical density was noted for 5 days and their percentage of decolourization was calculated and given in the Tables 5 and 6.

Table: 5 Dye decolourization under sterile condition

S.No	Organisms	Different concentration of bacterial cultures	Optical density					Pencentage of decolourization
			1st day	2nd day	3rd day	4th day	5th day	
1	*Pseudomonas* sp.	0.5 ml	0.58	0.47	0.40	0.38	0.29	50%
		1 ml	0.62	0.54	0.46	0.32	0.26	58%
		0.5 ml	0.50	0.43	0.32	0.28	0.23	54%
		1 ml	0.57	0.42	0.38	0.32	0.21	63%
3	Consortium of (*Pseudomonas* sp. + *Pseudomonas aeruginosa*)	0.5ml	0.72	0.63	0.56	0.42	0.33	54%
		1 ml	0.76	0.65	0.54	0.41	0.30	61%

Table: 5 Dye decolourization under unsterile condition

s.no	Organisms	different concentration of cultures	Optical density					Pencentage of decolourization
			1st day	2nd day	3rd day	4th day	5th day	
1	*Pseudomonas* sp	0.5 ml	0.63	0.53	0.44	0.38	0.32	49%
		1 ml	0.68	0.64	0.54	0.42	0.33	51%
2	*Pseudomonas aeruginsa*	0.5 ml	0.54	0.48	0.41	0.36	0.26	51%
		1 ml	0.63	0.52	0.40	0.32	0.25	60%
3	Consortium of (*pseudomonas* sp. + *pseudomonas aeruginosa*)	0.5ml	0.68	0.56	0.48	0.38	0.31	54%
		1 ml	0.75	0.67	0.52	0.45	0.32	57%

Discussion

Dye colour removal from spent textile dye mill is one of the consistent and persistent problem in the fields of environment. The removal of colour from waste is often more important than the removal of the soluble colorless organic substance, which usually contribute the major fraction of the biological oxygen demand (BOD). Treatment of the effluent by microbial degradation is an innovative method. In this study all physio-chemical parameters such as temperature, pH, colour, hardness, chloride, calcium, sodium, potassium, total, solids, total dissolved solids, suspended solids, and biological oxygen demand were estimated within the range, governed by pollution control committee. Based on typical colony morphology-large, opaque, irregular, smooth colonies and the biochemical characteristics such as Gram staining, motility,catalase test, IMVIC, casein hydrolysis, and glucose utilization the bacterial isoloates were identified as *Pseudomonas* sp. Synthetic dyes were widely found in the effluent of textile industries. The persistence and toxicity of these compounds cause adverse impact in receiving streams. So the degradation of dyes in textile effluent is important. To study the feasibility of biodegradation of the dye house effluent by employing the commercial strain *Pseudomonas stutxeri* under anoxic condition was carried out by other researchers. Based on this the comparative study was carried out to analyze the efficiency of the commercial strain *Pseudomonas aeruginosa* and the wild strain *Pseudomonas* sp. in different conditions (i.e. steile and unsterile) and in different concentrations (0.5 ml and 1 ml) and percentage of decolourization was calculated. The results showed that commercial strain *Pseudomonas aeruginosa* decolorized the dye effluent (63%) in sterile condition at 1 ml concentration more effectively than the wild strain *Pseudomanas* sp. (58%) in steile condition at 1ml concentration. Dyes have different molecular structures, the decolonization vary from one microbe to other microbe, so there was fluctuation in the percentage of decolorization among the strains (Keck et al., 1997).

Acknowledgments: The authors are cordially thankful to the Department of Biology, Gandhigram Rural Institute-Deemed University, Gandhigram for providing all essential facilities.

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