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Original Research Paper

Environmental Science

POTENTIAL OF BACTERIAL ISOLATES IN THE BIO-REMEDIATION OF TEXTILE EFFLUENT

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ABSTRACT

Aim and Objective: To determine the role of bacterial isolates on the physiochemical parameters of textile effluent.
Materials & Methods: The present study was focused on the estimation of physiochemical parameters like BOD, COD, Chloride, Calcium, Magnesium, Potassium, Phosphorus, Sodium, hardness, Sulphate and Nitrogen before and after treatment with the selected isolates from the effluent of textile industry.
Result: The results showed that there was a remarkable decrease in the physiochemical parameters when compared with the initial stage (i.e.) before treatment, especially with the treatment using Pseudomonas sp.
Conclusion: It is concluded from our study that the treated of textile effluent with bacterial isolates showed a remarkable biodegradation of the effluent with effective decline in the physiochemical parameters of the treated effluent to reduce environmental pollution

KEYWORDS : textile effluent, physiochemical parameters, bioremediation, biodegradation, environmental pollution

INTRODUCTION

Industrialization helps in the upliftment of the nation economy. However, releasing the industrial waste products into the environment has various issues. As these products creates threads due to low biodegradability and/or toxicity. Textile industries release nearly 10% of intensely coloured and toxic effluents with serious environmental pollution. The photosynthetic aquatic plants and algae are affected because of the release of dye which affect the absorption and reflection of sunlight. In order to reduce this pollution many eco-efficient biotechnological approaches were suggested by Wesenberg & Kyriakides, 2003. The textile industries produce effluents that contain chemicals like dispersants, acids, carriers, leveling agents, alkalis and dyes (Cooper, 1995). Discharge of millions liters of untreated effluents into public drains alters the pH, increases the biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and gives the rivers intense colourations (O'Neill et al., 1999). The removal of colour from textile waste water is often more important which usually contribute to the major fraction of Biochemical Oxygen Demand (BOD) by the fairly well established methods (Banat, Nigam, Singh, & Marchant,1996; Pearce, Lloyd, & Guthrie, 2003). On the other hand textile waste waters exhibit low BOD to COD ratios (< 0.1) indicating their difficulty to bioremediate or breakdown (Pagga & Brown., 1986). Several physiochemical methods are used in the treatment of textile effluents to achieve decolorization though these methods are effective yet quite expensive (Do, Shen, Cawood, & Jeckins, 2002.; Meyer 1981). Bio-treatment offers a cheaper and environmentally friendlier alternative for colour removal in textile effluents.

Materials and Methods

Effluent source:

The effluents were collected from textile industry in Namakkal district. The large suspended particles are removed through filtration by ordinary filter paper and stored at 4±1°C until use. The effluent sample was diluted with distilled water in the following concentrations.

S1- 2 ml of effluent was diluted with 8 ml of distilled water.
S2- 4ml of effluent was diluted with 6ml of distilled water.
S3- 6ml of effluent was diluted with 4ml of distilled water.
S4- 8ml of effluent was diluted with 2ml of distilled water.
S5- 10ml of effluent.

Isolation of Bacterial Culture from Effluent Sample:

Spread Plate Method: About 1.0ml from the serially diluted last dilution (10^{-} 9 dilutions) was spread on to the petriplate containing the molten nutrient agar were incubate them at 37° C for 24hrs and results were observed.

Streak Plate Method: The mixed colonies on the spread plate was selected and streaked on the nutrient agar plate to retrieve the pure isolate. The Petri plate was incubated at 37°C for 24hrs. After incubation the colonies were observed.

Isolation of Pure Culture: Single germinating spores were picked from the mixed culture containing several spores and subcultured. The purified cultures were transferred to nutrient medium and sub cultured was stored at 40 C until use.

Identification of isolates: Isolated strains were identified on the basis of their morphological and biochemical characteristics according to Bergey's Manual of Determinative Bacteriology.

Analysis of Physiochemical Parameters: The analysis for physico-chemical parameters in textile effluent sample was carried out before and after treatment according to the procedure outlined in standard method (Apha 1995).

Measurement of pH: About 50ml of effluent was taken in a beaker and the electrodes were immersed into the beaker containing the effluent and the meter readings were recorded.

Amount of total solids (TS): Accurately weigh a clean dry 100ml of silica crucible and record its weight as (WI). Then 5ml of unfiltered sample in the silica crucible was taken and evaporated by placing it in a hot-air oven at 1050c for 1 hour. Cool it in desiccators and then take the crucible out of the desiccators' and record its weight let it be (WF).

Calculation: $TS\,(mg/L) = \frac{W1 - W2}{S}$

Estimation of total dissolved solids (TDS): The total dissolved solids can be determined by evaporating and drying a known volume of the sample at 1800 C. The evaporating dish of suitable size was taken dried and weighed. The sample was filtered through What mans No:1 filter paper. Now the clear filtrate on the evaporating dish was evaporated in a water bath until constant weight was obtained. The dish was again weighed and the difference was noted.

Calculation: $DS\,(mg/L) = \frac{(A - B)*1000}{S}$

Where, A= Weight of the dried residue +dish (mg).
B=Weight of the dish (mg).

S=Volume of sample (ml).

Estimation of COD: To 50ml of sample 5ml of potassium permanganate was added and kept in a water bath at 100°C for one hour. Then cooled sample 5ml of potassium iodide and 10ml of concentrated Sulphuric acid were added. Then it was titrated against sodium thiosulphate until the appearance of pale yellow colour. To the resultant solution 1ml of starch was added and again titrated until the blue colour disappears.

Caculation: COD (mg/l) = 8*C (B–A) *1000
S

Where, C=Concentration of titrant.
A=Volume of titrant used for blank (ml).
B= Volume of titrant used for sample (ml).
S=Volume of water sample (ml).

Estimation of BOD: The BOD of the diluted sample in the first bottle was determined immediately after the sample collection and the other bottle was incubated for 5 days. Now the difference in DO was recorded. The sample (3.2ml of water and 122.8ml of phosphate buffer) was pipette out into a stopper bottle into which 2ml of manganese sulphate solution and alkali iodide solution were added. Then 2ml of concentrated Sulphuric acid was added along the sides of the bottle to dissolve the precipitate formed. The liberated iodide was titrated with the standardized thiosulphate in the burette and 2ml of starch was added. The titration was continued until the disappearance of straw yellow colour

Calculation: Dissolved oxygen (mg/l) = V2*N*8*1000
V1

V1=Volume of sample (ml).
V2=Titer value (ml).
N=Normality of thiosulphate

BOD (mg/l) = (D1–D2)
P

D1=Dissolved oxygen of diluted water sample immediately after the preparation (mg/l).

D2= Dissolved oxygen of diluted water after 5 days incubation (mg/l).

P=Volume of sample (ml) + Volume of dilution water added
Volume of sample (ml)

Estimation of Total Hardness: To 50ml of the well-mixed sample, 5ml of buffer solution, 1ml of sodium hydroxide was added and a pinch of eriochrome black T was added and titrated immediately against 0.01 M EDTA till the wine red colour changes to blue.

Calculation: Hardness (mg/l) = A*1000
S

Where, A=Volume of EDTA (ml)
S=Volume of sample (ml).

Estimation of Calcium: About 5ml of the well-mixed sample was taken in a conical flask, 2ml of sodium hydroxide, a pinch of indicator were added and titrated against EDTA till the pink colour changes to purple. The volume of EDTA consumed for calcium was noted down.

Calculation: Calcium (mg/l) = A*400.8 *1000
S

Where, A=Volume of EDTA (ml)
S=Volume of sample (ml).

Estimation of Sulphates: To 50ml of the sample, 10 ml of sodium chloride and hydrochloric acid solution, 1ml of glycerol-Ethanol solution and 150mg of barium chloride were added. The solution was stirred in magnetic stirrer for one hour. The absorbance was measured at 420nm. The standard was also treated in the same way. The amount of sulphate in the test sample was calculated from the standard graph.

Estimation of Nitrates: To 1ml of sample, 1ml of potassium nitrate and 4ml of propanol were added and incubated in dark for 30 minutes. The same constituents without effluent sample serve as control. About 1ml of sample was taken from the test and control were analysed. Different aliquate of potassium nitrate were pipetted out into a series of test tubes and made upto 1ml with distilled water. Then 1ml of sulphamilamide and Napthyl ethylene-diamine dihydrochloride were added to all the test tubes. The absorbance was read after 10 minutes at 540 nm .The concentration of nitrogen in the sample can be calculated from the standard graph.

Estimation of Inorganic phosphates: In 50ml volumetric flask 35ml of sample and 10ml Vanadate- molybdate reagent was added. A blank was prepared in which, 35ml of distilled water is substituted for the sample. After 10 minutes the absorbance of sample was measured against a blank at 400nm. Calibration curve was prepared by using suitable volume of standard phosphate solution.

Calculation: Phosphorus (mg/l) = phosphorus (mg) in 50 ml*1000
S

Where, S= Volume of sample (ml).

Estimation of sodium: The blank and sodium calibration standard (1.0 to 10.0 to 100mg/l) were prepared. A calibration curve was constructed from the sodium standards and sodium concentration of sample was determined from the calibration curve.

Calculation: Sodium (mg/l) = Sodium (mg/l)*D
D = Dilution ratio = S+A
S

A= Volume of distilled water (ml)
S= Volume of sample (ml).

Estimation of potassium: The Blank and potassium calibration standards (0 to 1.0 to 10.0 to 100mg/l) were prepared. A calibration curve was constructed from the potassium standards. And the Potassium concentration of sample was determined from the calibration curve.

Calculation: Potassium (mg/l) = Potassium (mg/l)*D
D= dilution ratio = S+A
S

A=Volume of distilled water (ml)
S=Volume of sample (ml).

Estimation of Magnesium: Magnesium (mg/L) = [Total hardness (mg/l) – calcium hardness (mg/l)]*0.243.

Results and discussion:

Isolation of microbes from Effluent source: In total, 130 isolates were collected from the Effluent sample and among them; two isolates (P1 and P2) were found to be dominative.

Table 1: Isolation of bacterial culture.

Sample	Dilutions	Number of colonies
Textile Effluent	10^{-5}	53
	10^{-6}	47
	10^{-7}	52
	10^{-8}	53
	10^{-9}	37

Morphological and biochemical characterization:

The suspected organisms were confirmed as Bacillus and Pseudomonas refer Table 2a Table 2b.

Table 2a: Characterization of isolates.

Tests	Isolate 1	Isolate 2
Color	Dull white	Dull white
Shape	Regular	Regular
Texture	Smooth	Smooth
Elevation	Undulate	Undulate
Margin	Convex	Convex

Table.2b: Biochemical Test.

S.No.	Biochemical tests	Isolate 1	Isolate 2
IMVIC Test			
1.	Indole production	-ve	- ve
2.	Methyl red	- ve	- ve
3.	Voges proskaur	- ve	- ve
4.	Citrate utilization	- ve	- ve
5.	Catalase	+ ve	+ ve
6.	Oxidase	- ve	+ ve
7.	Nitrate reduction	+ ve	+ ve
8.	Urease	- ve	- ve
Sugar tests			
9.	Glucose	- ve	+ ve
10.	Sucrose	- ve	- ve
11.	Lactose	- ve	- ve
12.	Mannitol	- ve	- ve
13.	TSI	+ ve	+ ve

The identified isolates were Bacillus and Pseudomonas

Preliminary examinination: The physiochemical parameters like BOD, COD, Chloride, calcium, magnesium, TS, TDS, hardness, phosphorus, potassium, sodium, sulphate, nitrogen were calculated refer Table.3 and results were tabulated. .

Table 3: Effluents before treatment.

S.NO	Physiochemical Parameters	S1	S2	S3	S4	S5
1.	Colour	0.169	0.224	0.250	0.2570	0.261
2.	TS	100	100	150	150	200
3.	TDS	2222	2277	2384	2486	2588.5
4.	COD	32000	40000	56000	72000	96000
5.	Chloride	710	1136	1597	2414	2627
6.	Hardness	300	533	833	866	933
7.	Calcium	16032	32032	48096	64128	96190
8.	Phosphorus	426	573	680	800	1000
9.	Magnesium	7580	7718	11484	11557	15380
10.	BOD	400	920	1140	1880	2400
11.	Potassium	0.012	0.014	0.017	0.018	0.019
12.	Sodium	0.0098	0.0112	0.0138	0.0154	0.016
13.	Sulphate	0.0031	0.0043	0.0054	0.0072	0.0079
14.	Nitrogen	0.003	0.0041	0.0053	0.0064	0.0073

Each value is measures of mean

Treatment after Bacillus: All samples after their initial examination were treated with Bacillus and the results were shown in Table 4.

Table 4 : Effluent after treatment.

S.NO	Physiochemical Parameters	S1	S2	S3	S4	S5
1.	Colour	0.083	0.091	0.10	0.110	0.117
2.	TS	100	100	100	100	150
3.	TDS	2141	2188	2272.5	2416	2486
4.	COD	11000	16000	32000	40000	48000
5.	Chloride	568	1065	1491	2059	2520
6.	Hardness	166	300	433	500	700
7.	Calcium	14428.8	16032	32032	48096	64128
8.	Phosphorus	400	546.66	666	786	960
9.	Magnesium	3879	5564	7580	7726	7759
10.	BOD	80	240	480	720	800
11.	Potassium	0.007	0.009	0.011	0.012	0.016
12.	Sodium	0.009	0.011	0.013	0.015	0.016
13.	Sulphate	0.001	0.0017	0.0029	0.0047	0.0059
14.	Nitrogen	0.0019	0.0021	0.0034	0.0037	0.0046

Each value is measures of mean

Treatment after Pseudomonas: Refer Table 5.

Table 5: Effluent inoculum after treatment.

S.NO	PHYSIOCHEMICAL PARAMETERS	S1	S2	S3	S4	S5
1.	Colour	0.063	0.081	0.097	0.101	0.109
2.	TS	50	50	100	100	150
3.	TDS	2215	2273	2326	2228	2425
4.	COD	8000	16000	24000	24000	40000
5.	Chloride	532	1029	1420	2059	2449
6.	Hardness	66	100	133	200	300
7.	Calcium	16032	32064	32064	16032	16032
8.	Phosphorus	266.66	386	533.33	643.33	853.33
9.	Magnesium	3839	3871	3875	7759	7767
10.	BOD	40	200	360	400	760
11.	Potassium	0.005	0.007	0.008	0.011	0.014
12.	Sodium	0.0036	0.0066	0.0084	0.0102	0.0150
13.	Sulphate	0.0022	0.0032	0.0049	0.0056	0.0067
14.	Nitrogen	0.0024	0.0032	0.0041	0.0051	0.006

Each value is measures of mean

DISCUSSION

Environmental pollution has been recognized as one of the major problems of the modern world. Organisms such as Pseudomonas and Bacillus were isolated from the effluent of textile industry. The effluent is also treated with the help of the above two organisms, is observed was that the physiochemical parameters results were declined when compared with that of initial stage (i.e.) before treatment. Thus, the ability of microorganisms to degrade and metabolize compounds has been recognized and exploited in the present study.

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