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Bioremediation ability of individual and consortium of non-immobilized and immobilized bacterial strains on industrial azo textile effluent

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ABSTRACT

This study was investigated the non-immobilized individual and consortium bacterial strains and immobilized individual and consortium bacterial strains for the reduction of azo dyes containing textile effluent. About 4 predominant bacterial strains such as Pseudomonas sp., Staphylococcus sp.,, Micrococcus sp., and Bacillus sp., with potential dye degradation ability were isolated from dye industries in tirupur. It was used to develop consortium for bioremediation efficiency analysis on textile effluent. On analyzing with the individual and consortium immobilized and non-immobilized bacterial strains of the treatment trials, the consortium non-immobilized bacterial strains are found to be the very effective bioremediation ability. This study reveals the optimization of pH, retention time, organic load, incubation time and Inoculums concentration for the effective decolorization of the azo dye containing textile effluent. The GCMS analyses of the treated (non-immobilized bacterial consortium) samples were not found to have any toxic compounds.

Keywords: non-immobilized bacterial consortium, Azo dye degradation, bioremediation, GC-MS, Physico-chemical parameters, immobilized bacteria.

INTRODUCTION

Industrialization is considered much important in terms of a country's economy [1]. But it could also be a threat in the environmental aspects of a nation. The toxic and hazardous compound exerted as a result of Industrialization could be major threat for the green planet. Highly colored substances are called as colorant. Soluble colorants are called as dyes and insoluble as pigment. These colorant and pigments could affect the several living creatures in earth. Dyes in water could cause water pollution. Ponds, river and oceans are now a day's get polluted by the textile dyes. Human metabolic and carcinogenic effects of dyes were reported by several studies. Dyes can be treated by physical, chemical and biological methods [2]. But the biological methods are considered to be best among them. Through there are several strains reported to degrade the dyes, still there are novel strains yet to be discovered which could be much more effective in dye degradation. Aromatic amines are formed after the degradation of azo dyes is main problem in anaerobic degradation and 60-70% of pollution is caused by the azo dyes. The azo dyes were degraded by the azo reductase enzymes which can produced by the bacteria [3]. The azo dyes can absorb sunlight which can harm plants and bacteria in water bodies. The individual bacterial strains are having less degradation

ability when compare to the consortium [4]. The individual bacteria can produce a small quantity of enzyme. In the case of consortium the enzyme production rate was high because more number of bacterial load [5]. The immobilized bacteria are having more stability than free system. The use of immobilized enzymes has significant advantages over soluble enzymes [6]. But the drawback was enzyme releasing to the textile water takes much more time when compared to the free system. The non-immobilized bacteria can directly produce the enzyme to the textile water [7]. So the enzyme starts to degrade the textile azo effluent. In the present study the efficiency of the individual and consortium non- immobilized and immobilized fungal strains were studied. The efficient organisms were then optimized under different cultural conditions to study the optimal bioremediative capacity.

MATERIALS AND METHODS

Sample Collection:
Azo textile effluent samples were collected from the dye industries in and around tirupur. This sample was used for isolating indigenous organisms and treatment process. The sample was brought to laboratory and stored at 7˚C.

Isolation by minimal agar medium with dye:
The minimal agar medium with dye is used for the isolating only the dye degrading microorganisms from the given sample. The samples were plated on minimal media with dye as a sole carbon source and required minerals were supplied. This method was used for further screening.

Screening of Selected Bacterial Isolates for Dye Decolourization:
Broth decolourization assay was done on nutrient broth amended with 0.01g of ten different dyes individually. The colonies which showed a growth on minimal agar media amended with dye were selected and inoculated in individual tubes for decolorization efficiency. The tubes were incubated at 27°c for 48 hours. The broth was checked for the color reduction in UV-Vis spectrophotometer after incubation [8].

$$\text{Percentage of decolourization} = \frac{\text{Initial absorbance - final absorbance}}{\text{Initial absorbance}} \times 100$$

Identification of Selected Microorganisms:
The screened bacterial strains from the broth decolourization assay which showed high reduction pattern were selected and identified by using standard biochemical and microscopic techniques [9].

Compatibility Test:
The antagonistic activity was checked by using Compatibility testing. The standard well-cut method was used to check the Compatibility of each strain on one another. Based on the zone of clearance the compatibility was assessed [10].

Characterization of Untreated Textile Effluent:
The raw effluent was characterized by measuring the values of 7 different physico-chemical parameters (TSS, TS, TDS, COD, pH, color, and turbidity) [11].

Treatment of textile effluent using Cultures immobilized and non-immobilized condition:
Textile effluent was treated using the Cultures on immobilized condition and non-immobilized strains [12]. The fresh log immobilized bacterial culture was added to the textile effluent. The samples were incubated in a metabolic shaker (120 rpm) for 24 hours at room temperature for a period of 5 days. Samples were retrieved from the flasks after 5 days of incubation and the bioremediation efficiency of the individual cultures as well as that of the consortium were studied by the physico-chemical parameters analysis. The same procedure was followed for non-immobilized bacterial strains for the textile treatment.

Comparative study of individual and consortium of immobilized and non-immobilized bacterial culture on dye degradation:
The comparative study was done by comparing the maximum bioremediation efficiency of the individual and consortium of immobilized culture and non-immobilized bacterial cultures, on the Azo dye containing textile effluent.

Optimization of Cultural Conditions for Maximum Bioremediation Ability:
The efficient combination of immobilized culture was selected and optimized under different parameters such as retention time, initial pH, incubation temperature, different substrate (dye) concentration.

The consortium of screened non-immobilized bacterial cultures was inoculated in the textile effluent. It was incubated for 24 hours at room temperature in a metabolic shaker. After the given incubation time the effluent sample was retrieved and the bioremediation ability of the consortium was studied for 5 days by measuring the various physico-chemical parameters (COD, TS, TSS, TDS, pH, Color and Turbidity). The optimum retention time, pH, incubation temperature, different substrate (dye) concentration for the consortium could be found out by measuring the reduction in the parameters during the specified period of incubation

GC-MS analysis
GC-MS has been widely used to identify products of dyes degraded with bacteria. The major limitation of this technique is that the sample must be volatile and thermally stable at the temperature of analysis. Identification of degradation products was made by comparison of retention time and fragmentation pattern [13].

RESULTS AND DISCUSSION

Isolation of Azo dye degradation Microorganisms:
The minimal agar medium with dye having growth of colonies showed the zone of clearance. It clearly indicates the growth of only the dye degrading microbial colonies. Among them only 21 predominant cultures were isolated [14].

Screening of Selected Bacterial Isolates for Azo Dye Decolourisation:
Among the 21 bacterial isolates selected strains 5, 9, 13 and 20 were found to be efficient against Azo dye by showing a reduction pattern of more than 58% [15].

Identification of Selected Microorganisms
The selected bacterial strains were identified as *Pseudomonas* sp., (isolate 5), S*taphylococcus* sp., (isolate 9), *Micrococcus* sp., (isolate 13) and *Bacillus* sp., (isolate 20) based on microscopic and biochemical characteristics [16].

Compatibility Test:
There was no zone of inhibition around the wells after incubation for any of the plates. The compatible nature of the bacterial strains shows that when used in a consortium it could produce enzymes in a wide spectral range that could be used in the reducing of the chromophore of the dye compound as well as the complex organic compound that is present in the effluent [17].

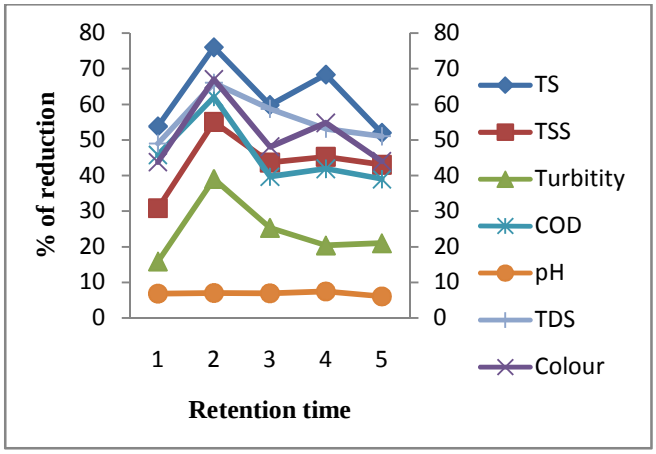

Figure 1: Effect of retention time by Non- immobilized bacterial Consortium on bioremediation

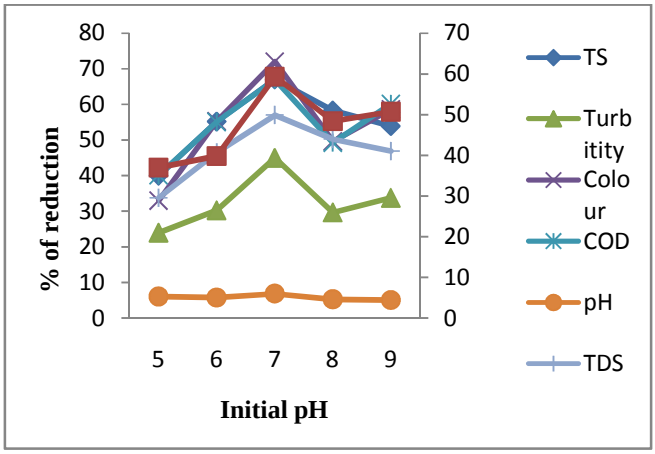

Figure 2: Effect of Initial pH by Non- immobilized bacterial Consortium on bioremediation

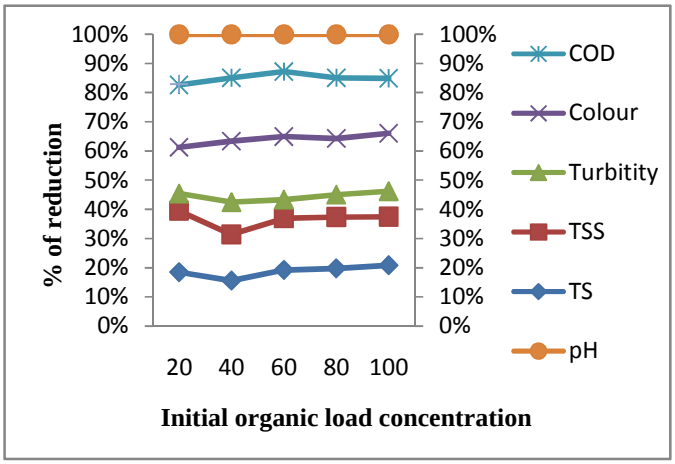

Figure 3: Effect of Initial organic load concentration by Non- immobilized bacterial Consortium on bioremediation

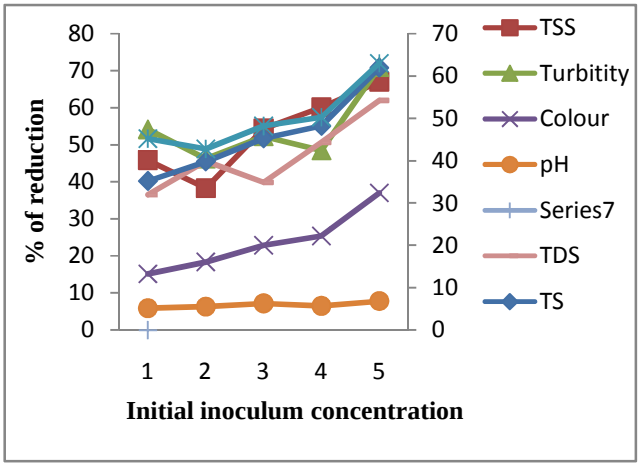

Figure 4: Effect of Initial inoculum concentration by Non- immobilized bacterial Consortium on bioremediation

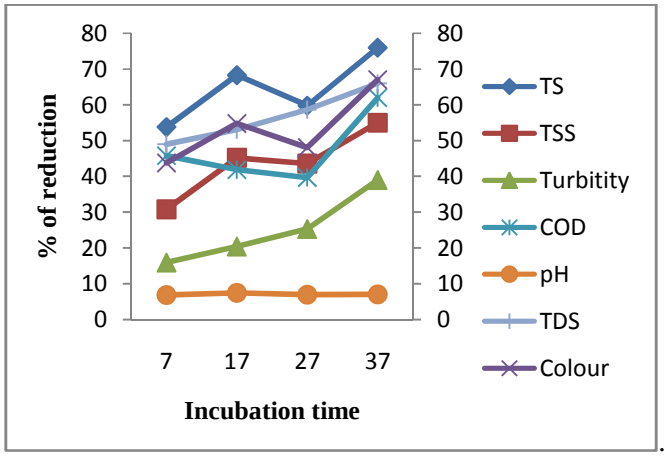

Figure 5: Effect of Incubation time by Non- immobilized bacterial Consortium on bioremediation

Physio-Chemical Characteristics Of Samples Retrieved After 5 Days Of Incubation

S.NO	Parameters	Treatment With non-immobilized Culture (5th Day)						Treatment With immobilized Culture (5th Day)					
		Initial Value	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Consortia	Initial Value	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Consortia
1	Colour	0.3240	0.2152	0.2834	0.1921	0.1606	0.1252	0.3240	0.2140	0.2815	0.2448	0.2945	0.2034
2	Turbidity	0.7696	0.4473	0.3882	0.5252	0.5918	0.2278	0.7696	0.6233	0.5710	0.7593	0.6620	0.5391
3	COD	920	660	710	595	540	400	920	860	770	730	820	700
4	TS	9200	6800	5200	4800	4000	2900	9200	6689	6021	7042	8191	5600
5	TSS	5500	2200	2600	1800	2000	1400	5500	3324	3848	3996	4390	2819
6	TDS	10000	8787	9886	6685	5486	4788	10000	9075	8208	9829	8379	7888
7	pH	7.63	7.93	7.99	7.90	7.86	7.27	7.63	9.72	8.99	8.14	8.79	8.90

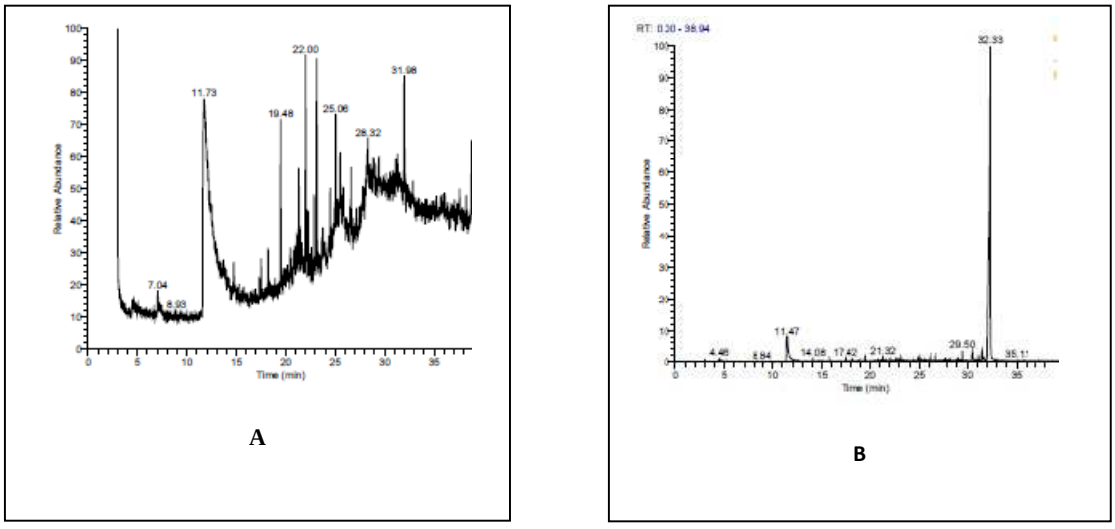

Figure 6: GC-MS chromatogram of untreated azo effluent (A) and effluent sample treated by the Non-immobilized bacterial consortium (B)

Treatment of Effluent Using Individual Cultures and Consortium of non-immobilized culture:

The initial values and the values obtained from 5th day were compared so that the maximum reduction in the sample for an immobilized and non- immobilized culture can be obtained. It is also useful to compare the efficiency between the samples treated with an individual culture or consortium. The physico-chemical characteristics of samples retrieved after 5 days of incubation is given in table. The consortium of non-immobilized bacterial culture is

shown very effective than individual non-immobilized bacterial culture and individual immobilized bacterial culture and consortium.

GC-MS analysis

The untreated textile effluent showed a number of peaks in its chromatogram. The compounds analyzed for these peaks were found to be the toxic product present in the untreated raw effluent sample and the treated effluent showed a major reduction in all the organic contents and the number of peaks that were observed was reduced to significant extent. The treated effluent were analyzed and found to be not toxic.

CONCLUSION

It could be concluded that, analyzing the chromatogram of the Non-immobilized treated and untreated effluent sample that the toxicity and organic load was reduced by the action of the Non-immobilized bacterial consortium as compared to the individual bacteria. This is mainly because of the wide spectral range and enzymes produced by the Non-immobilized bacterial consortium. The immobilized cells might be trapped to a certain extend so that it might not be efficient in degrading the dyes actively. The microbial consortium was not producing any toxic end products in reducing the dyes which could be carcinogenic to the system.

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