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Original Research Article

Biodegradation of Indigo Blue Dye using the Soil and Sludge Isolate of the Effluent Run off Site

C. Initha Lebanon Ebency[1]*, S. Rajan[2], A.G. Murugesan[3]

[1]*Department of Microbiology, Sree Narayana Guru College, K.G. Chavadi, Coimbatore–641105*
[2]*Department of Microbiology, M.R. College of Arts and Science, Manargudi, Tamilnadu, India*
[3]*Sri Paramakalyani Centre for Environmental Sciences, Manonmanium Sundaranar University, Tirunelveli, T.N, India*

A B S T R A C T

Cloths are of high demand from centuries as it implies the personality of a human being; hence the textile industry has established a wide empire over the time. Textile industries consume large volumes of water and chemicals during manufacture and processing. Colour is the first contaminant to be recognized in textile waste water and has to be removed before discharging into water bodies or onto land. Azo dyes account for 60–80 % of the dyes consumed in the textile processing. Indigo blue dye is used mostly in all jean manufacturing textile industries. To decolourise the indigo blue dye, the soil and sludge was collected from different jean manufacturing industries in Coimbatore district. Indigo blue dye degrading organisms were screened using Zhou and Zimmerman (ZZ) screening media after serially diluting the collected samples. After screening and isolating the best dye decolouriser from the samples, optimizations of the dye degrading conditions were carried out. Different factors like temperature, pH, carbon source, nitrogen source, metallic salts, inoculam size and time course of decolourization was selected as optimization parameters. The best degrader was found to be *Bacillus* sp. From the optimization analysis, the suitable optimized condition for the isolate, *Bacillus* sp. was identified as sucrose (1%), ammonium chloride (0.25%), temperature (45^{o} C), pH (8.0), potassium dihydrogen orthophosphate (84.96%) and inoculum dose (2%). The time required for effective decolourization (98%) of the indigo blue dye was estimated to be 48 hours. Thereby the results suggest *Bacillus* sp. isolated from the effluent discharge showed a potential of degrading the selected indigo dye at a faster rate and these properties are found to be useful for the bioremediation of textile industry effluent.

Keywords: Indigo blue dye, Bacillus sp. , Zhou and Zimmerman (ZZ) screening media, pH, carbon source, nitrogen source, metallic salts, inoculam size and time course of decolourization.

A R T I C L E I N F O

Contents

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***Corresponding Author**
C. Initha Lebanon Ebency
Department of Microbiology,
Sree Narayana Guru College,
K.G. Chavadi, Coimbatore–641105
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1. Introduction

Textile industries have a wide range of influence in pollution. Untreated effluents in the form of waste water are daily discharged from textile mills into public drains that eventually empty into rivers [1]. Azo dyes are mainly used because of the ease and cost effectiveness in synthesis compared to a natural dye. These dyes are toxic, carcinogenic and mutagenic in nature [2]. Azo bonds present in these compounds are resistant to breakdown, with the potential for the persistence and accumulation in the environment [3]. Effluents from the textile industries are the most expressive from an ecological and physiological perspective [4].

In order to achieve satisfactory and acceptable quality levels that allow recycling of textile waste water, removal of dyes and related compounds is very crucial. Based on the fact that azo dyes constitute the largest percentage of textile dyes, the most treatment methods are based on the decolourisation of azo dyes [5]. Currently the main operational methods used in treatment of textile waste water involve physical and chemical process [6] .All these physical and chemical methods are very expensive [7] and result in the production of large amounts of sludge, which creates pollution. Therefore, economic and safe removal of the polluting dyes is still an important issue.

Bioremediation through micro organisms has been identified as a cost effective and environment friendly alternative for disposal of textile effluent [8]. In recent years a number of studies have focussed on some micro organisms capable of degrading and absorbing dyes from waste water. A wide variety of microorganisms are reported to be capable of degrading and absorbing dyes from waste water [9]. Bacteria are known to degrade and mineralize many reactive azo dyes faster. The intermediate products synthesised during dye decolorization can also be degraded by hydroxylase and oxygenase produced by bacteria [10]. The azo dyes structures are reductively cleaved into colorless amines by several bacterial species. This behaviour is often seen in aerobic bacteria that grow in the presence of azo compounds. The intermediate sulphonated amines formed in this process may be aerobically degraded. The initial process in microbial degradation of azo dyes is the cleavage of highly electrophilic azo bond leading to decolourization of azo dyes. The reductive cleavage of the azo bond resulted in formation of aromatic amines as end products [11]. Dye waste water from textile and dyestuff industries is one of the most difficult industrial waste waters to be treated. Waste water from these industries is characterized by high alkalinity, biological oxygen demand, chemical oxygen demand and total dissolved solids. The synthetic origin and complex aromatic structures of dyes make them stable and persistant in the environment [12].

Considering the impact of dyes on environment, and the ability of microorganisms to metabolize azo dyes, in the present study an indigo blue dye used in jeans manufacturing textile industry was subjected to bacterial attack on optimized conditions. To find out the degrading ability of the indigenous flora the bacterial isolates were made from the textile effluent discharge sites.

2. Materials and methods

The entire research was carried out from February 2013 to September 2013. Soil and sludge samples were collected from different textile effluent discharge sites of Coimbatore district, Tamilnadu, India for screening the dye degrading bacterial isolates. A common dye used in jean manufacturing industry is indigo blue dye. This dye is selected for this study.

Methodolody

Isolation of dye degrading bacteria [13].

The dye decolourizing bacteria was isolated from the soil and sludge samples taken from the effluent run off site of the textile industry. The sample was serially diluted and plated on modified Zhou and Zimmermann (ZZ) agar medium. The serially diluted samples of 10^{-3} ,10^{-4} and 10^{-5} were plated on Zhou and Zimmermann (ZZ) agar medium and the plates were incubated at 37^{o} C for 24 hours. The incubated plates were observed for the predominant types of organisms. Different colony morphology representing each type of bacteria was observed on the plates. The isolates were further purified by streaking onto nutrient agar plates. The isolated cultures were enriched in an effluent basal medium. The inoculated effluent basal medium was incubated at 30 ^{o}C for 3 to 6 days in an orbital shaker at 120 rpm.

Evaluation of dye decolourisation

Decolourisation ability of each isolated colony was studied again by inoculating 0.02 g of indigo blue dye in 90 ml of the ZZ medium. 10%(v/v) inoculums of each isolate was inoculated and studied for decolourization separately. Uninoculated medium served as control. All the inoculated flasks were kept at 30 ^{o}C for 3 to 6 days in an orbital shaker at 120 rpm. About 2 ml samples were withdrawn aseptically and centrifuged at 8000 rpm for 15 minutes. The clear supernatant was used for measuring the absorption at 600 nm using UV –Vis spectrophotometer (Shmadzu, Japan).

The percentage of decolourisation was evaluated by using the formula

D = [(A0-A1)/A0] x100

Where

D, decolourisation in %

A0, initial absorbance;

A1, final absorbance

Selection and identification of dye degrading bacteria

Only the isolates that showed maximum decolourization ability was chosen for further study. The isolated organisms were based on microscopy, cultural characteristics and biochemical tests. During this analysis, all the isolates were identified only upto genera level.

Dye decolourisation optimization [14]

Decolourisation of indigo blue dye by the selected isolate was optimized with respect to the effect of 1% carbon sources (glucose, sucrose, lactose, mannitol), 0.25% nitrogen sources (peptone, yeast extract , ammonium sulphate , ammonium chloride), temperature (25°C, 37°C, 40°C and 45°C), pH(5-9), metallic salts (potassium dihydrogen orthophosphate. Magnesium sulphate, calcium chloride, Ferric chloride) and inoculums size (1-5%). Decolourisation experiments were carried out separately for each of these factors.

Time course of dye decolourisation [15]

The time course of decolourisation was carried out under optimum conditions obtained from the above studies. Indigo blue dye added media with optimized factors was inoculated with the best decolorizing isolate. To study the time course of decolourisation, inoculated flasks were incubated upto 144 hours. The samples were periodically analysed for the decolourisation activity for every 24 hours.

3. Results and Discussion

Nearly seven different isolates taken from the soil and sludge of the run off site were screened to degrade the indigo blue dye (Fig: 1). All the isolated organisms were studied for their degradation ability.

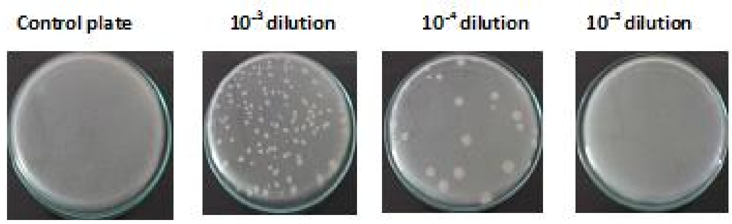

Figure 1: Isolation of dye degrading bacteria (Indigo Blue colour dye)

Among the seven isolated cultures DD4 showed maximum degradation ability on the selected indigo blue dye (Table: 1). Based on the biochemical analysis the isolate DD4 was identified as *Bacillus* sp. (Table: 2).

Table 1: Decolourisation efficiency of the isolated cultures

S.No	Dye degraders	Percentage of degradation for indigo blue colour dye (%)
1	DD1	65
2	DD2	81
3	DD3	73
4	DD4	98
5	DD5	77
6	DD6	68
7	DD7	79

Table 2: Identification of the best degrading organism DD4

S.No	Biochemical tests	DD4
1	Indole test	Negative
2	Methyl red test	Negative
3	Voges-prousker test	Positive
4	Citrate test	Positive
5	Oxidase test	Negative
6	Catalase test	Positive
7	Urease test	Negative
8	Starch hydrolysis test	Positive
9	Gelatin hydrolysis test	Negative
10	Casein hydrolysis test	Negative
11	Nitrate reduction test	Negative
12	Carbohydrate fermentation test	Negative

From the optimization analysis (Table: 3), the suitable optimized condition for the isolate, *Bacillus* sp. was identified as sucrose (1%), ammonium chloride (0.25%), temperature (45^{o} C) , pH (8.0), potassium dihydrogen orthophosphate (84.96%) and inoculum dose (2%).

Table 3: Optimization of different parameters for potential degradation

	Carbon source	**OD of control**	**OD of sample**	**Percentage**
Indigo Blue dye	Glucose	1.557	.275	82.33%
	Sucrose	**1.268**	**.214**	**86.25%**
	Lactose	1.046	.319	76.65%
	Mannitol	1.595	.297	80.92%
	Nitrogen source	**OD of control**	**OD of sample**	**Percentage**
Indigo Blue dye	Yeast extract	1.041	.143	86.26%
	Peptone	1.046	.138	86.80%
	Ammonium sulphate	1.056	.120	83.02%
	Ammonium chloride	**1.096**	**.186**	**88.63%**
	Temperature	**OD of control**	**OD of sample**	**Percentage**
Indigo Blue dye	25ºC	1.110	.365	67%
	37ºC	1.149	.503	56.22%
	40ºC	1.116	.166	85.12%
	45ºC	**1.213**	**.089**	**92.66%**
	pH	**OD of control**	**OD of sample**	**Percentage**
Indigo Blue dye	5	1.144	.244	78.67%
	6	1.323	.351	73.46%
	7	1.295	.259	80%
	8	**1.089**	**.139**	**87.23%**
	9	1.038	.137	86.80%
	Inoculum size	**OD of control**	**OD of sample**	**Percentage**
Blue	1%	1.138	.123	89.19%
	2%		**.099**	**91.30%**
	3%		.114	89.98%
	4%		.119	89.54%
	5%		.165	85.50%
	Metallic salts	**OD of control**	**OD of sample**	**Percentage**
Indigo Blue dye	**Potassium dihydrogen orthophosphate**	**1.024**	**.154**	**84.96%**
	Magnesium sulphate	1.019	.172	83.12%
	Calcium chloride	1.015	.185	81.77%
	Ferric chloride	1.009	.192	80.97%

Furthermore many studies revealed the similar results indicating the effective degradation of the textile effluent using *Bacillus* sp.[(16)].The factors such as temperature, pH, metallic salts, carbon and nitrogen sources play a vial role in mediating the degradation ability of a micro organism [(17)]. The time required for effective decolourisation of thesample was analysed and it was found out that

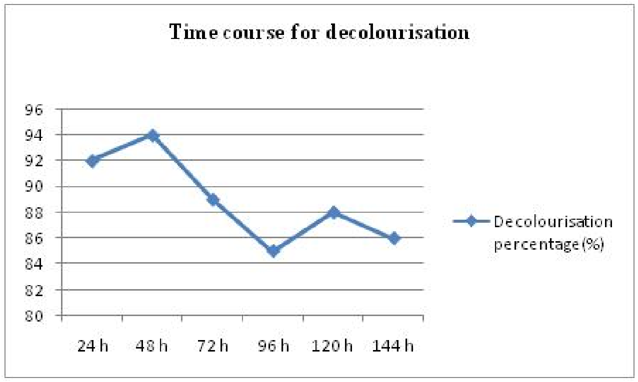

Figure 2: Time course for effective decolourisation of the dye indigo blue

4. Conclusion

Textile industries had faced the problems related to effluent discharge into the public accessing water bodies and aquatic sources for the past several years. The dye and its concentration in the effluent was reported to present different chemical compounds which may be carcinogenic to human and animal populations when the contaminated water was consumed. Several researchers have contributed to decolourize or degrade the dyes and its constituents for past two decades. In the present research one such dye causing the similar problem in Coimbatore district was selected and its decolourisation was measured using the strain isolated from the effluent discharge site itself. Interestingly, as expected under optimized conditions, good decolourisation activity was measured for the isolate at a faster rate.

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