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BIODECOLOURIZATION OF AZODYE (PIGMENTED RED 208) USING *Bacillus firmus* AND *Bacillus laterosporus*

K. Saraswathi [1]*and S. Balakumar[2]

1. Department of microbiology, Sri Sankara Arts and Science College, Asur, Kumbakonam.
2. Department of Chemistry and Biosciences, SRC, SASTRA University, Kumbakonam – 612001, Tamil Nadu, India.

e-mail: saraswathi.kesavan@gmail.com

Abstract:

The textile industry in India is one of the oldest industries. It provides direct employment to nearly thirty million people. Waste water from textile industries are a complex mixture of many polluting substances such as organo chlorine based pesticides, heavy metals, pigments and dyes. The dye that has been chosen for this study is the monoazo dye. Some of the azo dyes, xanthene dyes and anthroquinone dyes are known to be very toxic and mutagenic to living organism. These chemicals alter the physio - chemical parameter of the water (pH, Biological Oxygen Demand-BOD, Chemical Oxygen Demand-COD). Decolourization and degradation of azodyes can detoxify the effluent. Biodegradation are environmental friendly and the cost competitive alternative to chemical decomposition processes. The present study focused the isolation of potent species from soil and there by decolourization of pigmented red 208 (HF2B), widely used in textile industry in India.

Key words:

textile effluent, azodyes, *Bacillus firmus* *Bacillus laterosporus*, decolourization.

1. INTRODUCTION

Pollution has been defined in various ways. It is considered as the release of unwanted substances to the environment by man in quantities that damage either the health or the resources itself. Water pollution involves the release of small amounts of substances directly through point sources or indirectly through non point sources. Industrial effluents from various industries like textile, dye stuffs, paper and pulp, distillery, olive oil mill and metal industries are the major contributor to water pollution.

Waste water from textile industries are a complex mixture of many polluting substances such as organo chlorine based pesticides, heavy metals, pigments and dyes. The dyestuff usage has been increased day by day because of tremendous increase of industrialization and man's urge colour [10]. Many chemical dyes have been used increasingly in textile and dyeing industries because of their ease and cost effectiveness in synthesis, firmness and variety in colour compared to that of natural dyes. About 100,000 commercial dyes are manufactured including several varieties of dyes such as acidic dye, basic dye, reactive dye, azo dye, and diazo dye. Colour is one of the most obvious indicators of water pollution and discharge of highly coloured synthetic dye effluents can be damaging to the receiving water bodies [12]. The effluents of pharmaceutical, textile, printing, photographs, and cosmetics contain dyes [9].

The dye that has been chosen for this study is the monoazo dye. The name azo comes from "azote", an old name of nitrogen that originates in French and is derived from the Greek a (not) + Zoe (to live). Azodyes are considered as electron deficient xenobiotic compounds because they possesses the azo (N=N) and sulfonic group ($-So_3^-$) electron withdrawing groups, generating electron deficiency in the molecule and making the compound less susceptible to oxidative catabolism by bacteria and fungi. Most of the textile industries that is 60 – 70% used azodyes for dyeing and other purposes. Some of the azo dyes, xanthene dyes and anthroquinone dyes are known to be very toxic and mutagenic to living organism.

The world annual production of dye stuffs amount to more than 7×10^5 tones [16]. As per the recent data published by the Textile commissioner's office, there are 1569 cotton textile industries in India except the small scale industries. India dye industries produces every type of dyes and pigments, in this production is close to 80,000 tones and India second largest exporter of dye stuff and intermediates developing countries after China.

Dyes with striking visibility in recipients may significantly affect photosynthetic activity in aquatic environment due to the reduced light penetration and may also be toxic to some aquatic lives due to the effluents containing several types of chemicals such as dispersants, leveling agents, acids, alkalis, carriers and various dyes [6].

The waste water discharge from textile and dyestuff industries have to be treated due to their impact on water bodies and growing public concern over their toxicity and carcinogenicity.

Currently the major methods of textile waste water treatment involve physical and chemical process. There is also possibility that a secondary pollution problem arise because of excessive chemicals used. Other emerging techniques, such as ozonation, treatment using Fenton's reagent, may have potential for decolourization. However, such technologies usually involve complicated procedures or economically unfeasible. The drawback of physical and chemical method treatment are oxidation is one of the physical treatment used in waste water its have high energy and formation of hazardous by-products. Another physical method is adsorption, it requires regeneration or disposal. Chemical methods are membrane technologies and coagulation produced high concentration of sludge production during treatment.

Alternative approaches to colour removal utilizing microbial catalysts to reduce the dyes that are present in the effluent. Microbial decolourization and degradation is an environment friendly and cost competitive alternative to physical and chemical degradation processes [18].

Recent fundamental work has revealed the existence of a wide variety of micro organisms capable of decolourising a wide variety of dyes. Many microorganisms belonging to different taxonomic groups of Bacteria, Fungi, Actinomycetes and Algae have been reported for their ability to decolourise azo dyes. Bacterial degradation of these dyes requires by their intracellular uptake while the fungi degrade these by extracellular enzymes [19]. Recently number of studies focused the some bacteria and fungi, which are able to biodegrade and bioadsorb the dyes in textile industry effluent [15]. The organisms used in most of the study were *Staphylococcus* sp, *E.coli*, *Bacillus* sp, *Clostridium* sp, and *Pseudomonas* sp in bacteria [8].Microbial decolourization of azo dyes has been reached by sequential anaerobic and aerobic conditions [7].

The main aim of this study of this study was to investigate dye decolourising potential of a consortium isolated from contaminated soil of an industrial estate and provides biologically favorable environment required to accomplish azodye decolourization in an effective manner using Bacteria. The present study focused the isolation of potent species from soil and there by decolourization is one of the interest areas in biological aspect of effluent treatment. The azodyes selected for this study was pigmented red 208 (HF2B), widely used in textile industry.

2. EXPERIMENT

2.1. Materials

2.1.1: Collection of samples

Dye house effluent, dyes, and soil (washing sites of near industry) were collected from an Easwari dyeing unit in Erode, Tamilnadu, India. They were stored in refrigerator at 4^0c and used without any pretreatment.

2.1.2: Dyes and chemicals

The dye used in this study was pigmented red

208 (HF2B). It was a monoazo dye, molecular formula of this dye was $C_{29}H_{25}N_5O_5$ and structural formula was 2-[[3-[[(2, 3-dihydro-2-oxo-1H benzimidazol-5-yl)amino] carbonyl]-2-hydroxy-1-naphthyl] azo] benzoate. The structure of pigmented red 208 was mentioned in figure-1.

All other chemicals used were the highest purity available and analytical grade.

2.1.3: Growth medium

The isolated microbial consortium and decolourization study were carried out in Mineral salt medium (MSM) of following composition (g/L): Na_2HPo_4:3.6, $(NH_4)_2$ So_4: 1.0, KH_2PO: 1.0, $MgSo_4$: 1.0, $Cacl_2$: 0 .10 and 10ml of trace element solution per liter was used for all the studies. The trace element solution used was had following composition (mg/L): $ZnSo_4$: 1 $Mncl_2$: 3 $Nicl_2$: 2 Na_2moO_4 : 3 H_3BO_3 :30 $Cucl_2$: 1.The final pH of the medium was adjusted to 7.0. The MSM was supplemented with 0.1% (w/v) of each yeast extract and glucose. The yeast extract, glucose and dyes were added to sterilize MSM from their respective filter stock solution [8].

2.2: Methods

2.2.1: Analysis of physiochemical parameters

The collected textile dye effluent physio chemical parameters of pH, colour, odor and COD were measured using standard methods .

2.2.2: Isolation and screening of dye decolourising microorganism

The collected soil samples were used for screening of dye decolourising cultures by enrichment technique using MSM amended with dye (HF2B- pigmented red 208, 100mg/L) as a sole source of carbon and energy or along with glucose and yeast extract. Dye containing media (100ml) in 250ml Erlenmeyer flasks were inoculated with 10ml soil suspension (10%w/v) and incubated in orbital shaker under static condition (150rpm). After 48 hours of incubation, 1.0ml of the culture broth was appropriately diluted and plated on MSM agar containing 100mg/l HF2B. After incubation morphologically distinct bacterial isolates showing clear zone around their colonies due to the decolourization of dye, were selected for further studies. The pure culture stocks stored at 4^0c on potato dextrose agar slopes [11].

2.2.3: Identification

A pure colony of the unknown isolates were named as B1 and B2, these isolates were identified presumptively on the basis of the following features gram staining, motility and biochemical reaction [2].

2.2.4: Dye decolourization studies in liquid medium

Frequently used dyes were collected from dye house industry and one colour was selected for better decolourization study using isolated bacterial organism.

2.2.4.1: Inoculation of bacterial biomass

The identified organisms B1 and B2 were grown in nutrient broth. About 5% (V/V) of 18 hour old culture was added for decolurization studies [3].

2.2.4.2: Decolourization experiment

The MSM medium constitutions amended with 100ppm of HF2B-pigmented red 208 was inoculated with bacterial culture (5% v/v) and incubated at 37°C under static condition. Samples were withdrawn at a particular interval time (not exceed 7days) and analyzed for decolourization [11].

2.5.4.3: Study of physio chemical parameters

Decolourization was studied using various carbon and nitrogen sources. The carbon and nitrogen sources used for this study were glucose, sucrose (carbon source) and yeast extract, ammonium sulphate (nitrogen source). The concentration supplemented sources were 1% (1g/100ml) [17].

2.5.4.4: Analysis of decolourization rate

The decolourization rate was monitored spectrometrically. The absorbance was monitored at 528nm, after incubation 5ml culture was taken from flask and centrifuged using bench top centrifuge (5000rpm for 20 minutes). All experiments were carried out in triplicates and the mean value was taken. Medium without dye and inoculum was used as blank. Medium with dye but without inoculum was used as control. The decolourization efficiency was expressed as per the following equation or formula [14].

$$\text{Decolourization }\% = \frac{I - F}{I} \times 100$$

Where,

I : initial absorbance

F : final absorbance

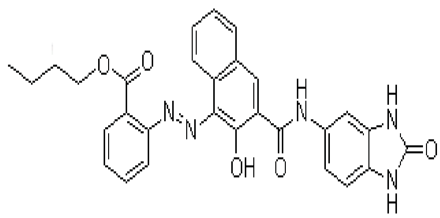


Fig1: Structure of HF2B, λ_{max} -528nm

3: Results and discussion

3.1: Isolation of dye decolourising bacteria

Isolates from contaminated soil samples were carried out by the enrichment culture technique using pigmented red as a sole source of carbon and energy. But we were not successful in isolating bacteria capable of decolourising and utilizing only dyes as a sole source of carbon and energy. So we were supplementing the glucose and yeast extract for isolate and screening the micro organism.

Decolourization occurred only when a carbon and energy source were available in the growth medium, glucose and yeast extract along were employed as co substrates, as a requirement for a metabolizable carbon sources seems to be obligatory for functioning of dye decolourizing bacteria [12]. About five different morphologically distinct bacteria isolated from the soil. Among them two bacterial isolate showed higher decolourization and it was used for further study. The two isolates were identified as *Bacillus firmus* and *Bacillus laterosporus* based on the gram staining, motility and biochemical reaction mentioned in [2] and the results were tabulated on Table1.

Abdulrahim et *al* and Chen *et al* were reported organism isolated from the site near textiles industry complex because of micro organism to survive in the presence of toxic dyes. Moofsi et al. [17] reported the *Paenibacillus polymyxa* (AY302489), *Micrococcus luteusi (AJ409096)*, and *Micrococcus* sp (AJ313029) were isolated from soil. *Steenotrophomonas acidaminiphila, Pseudomonas putida, Pseudomonas fluorescence* and *Bacillus cereus* were the isolated organism reported by Khehra *et al*. *Aspergillus* sp had a potential effect on the decolourization was reported in [11] mohandass *et al*.

3.2: Decolourization study by isolated strains

3.2.1: Decolourization of azodyes (pigmented red-208) in liquid medium

The isolated and identified organisms were grown in appropriate liquid medium; it was mentioned in materials and methods. The inoculation biomass was prepared and poured in MSM amended with pigmented red-208 (100ppm). In this step only studied the decolourization ability of isolated organism on MSM broth without any supplementations, then it incubated under shaking condition because Moosvi *et al* reported the decolourization up to 93% within 38 hours under shaking condition. After incubation decolourization assay was performed as per the formula. The decolourization rate of *Bacillus firmus* and *Bacillus laterosporus* were showed in Table: 2.

But good results were not uptained after 7days of incubation *Bacillus firmus* decolourized the dye only19.04% and *Bacillus laterosporus* decolorized the pigmented red -208 only 9.5%. In the absence of any supplementation (glucose, yeast extract etc.,) bacterial consortium showed lesser decolourization (40%) which indicates the necessity of supplementary source for growth and decolourization of dye was denoted by [12].

Table: 1 :Physiological and biochemical characterization of isolated strains

S.No.	Morphological and biochemical characteristics of isolates	*Bacillus firmus*	*Bacillus laterosporus*
1	Gram staining	+	+
2	Motility	-	+
4	Morphology	Rods	Rods
5	Spore staining	+	+
3	Indole test	-	-
4	Methyl red test	+	-
5	Voges proskauer test	-	-
6	Citrate utilization test	+	-
7	Urease test	+	+
8	Triple sugar iron test	Acid bud , alkaline slant	Acid bud , alkaline slant
9	Nitrate reduction test	+	-
10	Starch hydrolysis	+	-
11	Glucose fermentation	Acid production no gas	Acid production no gas
12	Voges proskauer test at pH<6	-	-
13	Growth at 50°C	-	-
14	Growth under anaerobic condition	-	-

3.2.2: Effect of various sources on decolourization.

The ability to decolourize RV5R in presence of other carbon sources was tested to obtain efficient and faster decolourization obtained .Glucose showing maximal decolourization compare with other sources. Moreover the ability of the culture presence of the culture to decolourize RV5R in the presence of different nitrogen sources other than yeast extract was studied. Only yeast extract showed highest decolourization rate compared with other nitrogen sources [3]. The selected isolates were checked for their ability to decolourize AR-88 (20mg/l) in MSM broth. The isolates named as *Stenotrophomonas aciaminiphila*, *Pseudomonas putida*, *Pseudomonas fluorescence* and *Bacillus cereus* it decolourized 78%, 80%, 85%, and 89% respectively. Glucose is essential for decolourization of AR-88, as in the absence of glucose only 20% decolourization of the added dye was achieved in 24h. Decolourization efficiency of the consortium increased. Yeast extract was found to be the most effective supplement for supporting higher decolourization efficiency. In the absence of yeast extract supplemented in MSM, only 34% of colour removal was observed whereas complete decolourization of the dye was achieved at 0.1% of yeast extract in medium in 12hours [8]. In this study results were optained when supplementing the carbon and nitrogen source decolourization rate was considerably increased and

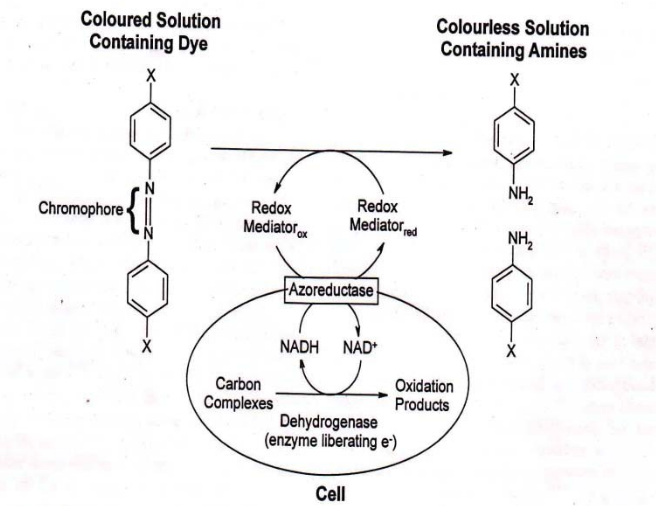
Table: 2 Decolourization study with isolated bacteria

S.No.	Organism isolates from soil	Decolourization rate of isolates with different sources on Pigmented red -208 (%)				
		MSM	Glucose	Sucrose	Yeast extract	Ammonium sulphate
1	Bacillus firmus	19.04	70.90	60.15	88.66	20.02
2	Bacillus laterosporus	9.5	82.4	60.8	90.95	21.06
3	Decolourization time	7	4	5	4	6

Table: 3 : Physiochemical analyses of textile effluent

S.No.	Physiochemical property	Untreated effluent	Treated with *Bacillus laterosporus*
1	pH	12.4	7.5
2	Decolourization rate%	0 (dark red)	80
3	COD mg/L	753.2	113.39

Fig 2: Reaction occur during azo dye degradation



decolourization time also reduced the all data were denoted in Table: 2. Based on the results *Bacillus laterosporus* decolurization rate was increased, and the time also decreased, when added glucose and yeast extract in the medium (separately) the data were 80.4% and 90.95% respectively. But *Bacillus firmus* not given a good result compared with *B.laterosporus* decolourization (Table:2) study. Hence the *B.laterosporus* with the potential use in effluent treatment, it decolourize and detoxify the crude textile effluent efficiently, the results were tabulated in Table: 3.The pH, COD, and colour were reduced from pH 12.04 to 7.5, COD from 753.2mg/l to 113.39mg/l and decolourization rate was 80%.

3.2.3 Mechanism of colour removal

Fig: 2 show a proposed mechanism for the redox mediator dependent reduction of the azo dyes using whole bacterial cells, under aerobic conditions. Although the final reduction of the a zo dyes in the cell supernatants is a dominantly chemical redox reaction, the redox mediators depend on cytoplasmic reducing enzymes to supply electrons [20]. It is also possible that this chemical redox reaction works in conjunction with a direct enzymatic reaction involving an azo reductase, which may be a dehydrogenase enzyme that is synthesized throughout the cytoplasm and secreted without accumulation inside the cell [4].

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