JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2010. Vol. 4(2), p. 617-622

Decolorization of Textile Sulfonated Azo Dyes by Bacteria Isolated from Textile Industry Effluent, Soil and Sewage

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(Received: 09 January 2010; accepted: 18 February 2010)

Biological oxidation of organic dyes is important for textile industry wastewater treatment. Decolorization of four textile sulfonated azo dyes (Direct Blue 71, Direct Yellow12, Acid Black 210, and Reactive Black 5) by six bacteria IE1 (*Citrobacter freundii*) , IE2*(Xenoranadus luminescence*) and IE3 (*Enterobacter cloace*) IE4 and IS1(*Pseudomaonas species)* ISEW *(Enterobacter agglomerans* Aerogenic strain Biotype-G2) isolated from two textile industry effluents, soil and sewage was studied. These isolates showed significant dye decolorization with a percent decolorization in range of 66.1% to 93.9% **(Direct Blue 71),** 26.73% to 92.46% **(Direct Yellow12),** 39.6% to 91.6% **(Reactive Black 5), and** 27% to 87.7% **(Acid Black 210) under strict aerobic condition, at room temperature (RT) and neutral pH within 24h.**

Key words: Textile industry, Azo dyes, Bacteria, Soil & Sewage.

Synthetic dyes have a wide application in the food, pharmaceutical, textile, leather, cosmetics and paper industries due to their ease of production, fastness, and variety in colour compared to natural dyes.

More than 10,000 synthetic dyes are commercially available and close to one million tons of these dyes are produced annually worldwide [1](Adedayo et al., 2004). Azo dyes, the largest group of dyes used in textile industry[2],constitute 60-70% of all dyestuffs produced[3] and consumed [4].They have aromatic rings with one or more azo groups (R1-N=N-R2) substituted mostly by sulfonate groups[3,5].

The substituted rings of these dyes are responsible for intense color and water solubility. But these dyes are resistant to degradation under conventional wastewater treatments [6]. Textile industries consume a large amount of water during dying [7] and consequently generate an equally large quantities of coloured effluent [8].

One of the environmental problems faced by textile industry is the removal of color from

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this dye wastewater prior to discharge to local sewage treatment facilities or adjoining water courses [9,10]. Color in the effluent is one of the most obvious indicators of water pollution and the discharge of highly colored synthetic dye effluents is aesthetically displeasing and can damage the receiving water body by impeding penetration of light. Moreover, azo dyes as well as their breakdown products are cytotoxic or carcinogenic [11]. A number of physico-chemical methods, such as adsorption, coagulation, precipitation, filtration and oxidation, have been used to treat dyestuff effluents, but these methods have many disadvantages and limitations [12]. It is, therefore, important to develop efficient and cost-effective methods for the decolorization and degradation of dyes in industrial effluents and contaminated soil [13]. In the present study, four azo dyes, Direct Blue 71(DB71), Direct Yellow 12(DY12), Acid Black 210(AB210), and Reactive Black 5(RB5) were selected for microbial dye decolorization by bacterial isolates for the textile industry Effluent (TIF), soil and sewage.

MATERIAL AND METHODS

Chemicals

Commercially important and commonly used azo dyes for textile dyeing DB71, DY12, AB210 and RB5 were obtained from local markets.

All other chemicals used were of analytical grade.

Enrichment, Isolation and cultivation of bacteria efficient in decolorization

Two textiles industry effluent samples were obtained one from Vasai, district Thane, Maharashtra and other from Gujrat, soil sample from garden, and sewage sample.

All the samples were stored at 4^0C until use.

All bacterial isolations were carried out in Minimal medium (MM), Bushnell and Hass

Mineral medium (BHM)[14] and Nutrient broth (NB) [15] but because of the fact that the best results for decolorization were obtained in NB, containing (gl^{-1}) peptone 10, NaCl 5, meat extract 0.3 and dye (DB 71) 0.1 was selected in the study. The textile industry samples (1ml), soil suspension (0.1g/10ml w/v)1ml and sewage 1ml were inoculated into respective labeled 250 ml flasks containing 100 ml NB medium and dye (DB71). Each flask was incubated at RT on a rotary shaker until decolorization was observed. Three Successive enrichments were carried out by inoculating 1ml from the decolorized media in fresh sterilized 100 ml BHM, MM, and NB containing 100mg l^{-1} dye DB71. After third successive enrichment mixed bacterial culture was streaked onto NB agar containing 100mg l^{-1} DB71 dye. Separate colonies of the predominant type of microorganisms were purified by re-streaking on the same medium. The purified isolates were examined microscopically to check their purity. Obtained pure cultures were maintained on NB agar at 4^0C (in refrigerator)[16,10,17].

Identification of Selected Dye Decolorizing Bacteria

A pure colony of the isolate was identified presumptively on the basis of the following

Features : colony morphology, colonial pigmentation, cell morphology, Gram-staining, and biochemical tests as per the *Bergey's Manual of Determinative Bacteriology 9th Edition.*

Screening for the most efficient dye degraders on the basis of percent decolorization

This experiment was performed in two sets.

In the first set 18 h old culture was scrapped from NA slants and a cell suspension of density 10^8cells/ml was prepared by adjusting absorbance$_{530nm}$ to 0.1ODU. Flasks containing NB with dyes DB71, DY12, AB210 and RB 5 at a concentration of 100mgl^{-1} were inoculated separately with 0.1 % of culture whereas in the second set culture was previously adjusted to medium containing 100mg l^{-1}dye. The decolorized medium was centrifuged and the pellet obtained was washed thrice with sterile saline, culture suspension of 0.1 absorbance at 530nm was prepared and inoculated in four flasks containing NB with each separately DB71, DY12, AB210 and RB5. Both the sets were incubated on rotary shaker at 150 rpm and RT along with controls that were without inoculum. Small aliquots were removed after 24 and 48 h, centrifuged at 3000 rpm for 30 min. supernatant was collected and absorbance was checked spectrophotometrically at λ_{max} of respective dye. The experiment was

performed in triplicates and the percentage of decolorization was calculated using following formula:

$$\% \text{ decolorization} = (A_0 - A_t / A_0) \times 100.$$

where

A_0 = initial absorbance

A_t = final absorbance

RESULTS AND DISCUSSION

Enrichment, Isolation and cultivation of bacteria efficient in decolorization

Bacteria decolorizing DB71 dye were enriched from textile industry effluent, soil and sewage sample by successive enrichment technique. There have been some reports which suggest that decolorization of certain sulfonated azo dyes occur under aerobic conditions after 2 months [18]. Another report indicates that azo dyes are essentially non-degradable by bacteria under aerobic conditions [19] .Hence present work was carried out to enrich and isolate organisms decolorizing sulfonated azo dyes under aerobic conditions.

Three media, BHM, MM and NB were used for enrichment under aerobic conditions. Dye decolorization was observed for DB71dye in 24h only in NB medium and not in BHM and MM. Ozdemir *et.al*. (2006) [14] carried out a similar work of isolation of bacteria decolorizing azo dyes in LB, BHM and MM but the best results for decolorization were obtained in LB medium, containing (g l^{-1}) 0.05 yeast extract, 0.1 tryptone, 0.1 NaCl , from a leather industry waste water and a yeast industry waste water. In the present paper medium NB containing peptone, meat extract, and sodium chloride was found to be the best medium for decolorization rather than BHM and MM. This result corroborate the results of Kodam *et.al*.(2006) [20] This indicates that the bacteria present in all the samples were able to degrade the dye in NB and not in the medium containing only salts. As NB contains the complex nutrients such as meat extract and peptone, one of the component of these may be required by the bacterium for dye degradation. The reason for this is that the bacteria may need a co substrate for the degradation of xenobiotic compounds like azo dyes**.** In the reference of Stolz (2001) [21] the

Table 1. Percent decolorization by non-adapted bacterial isolates after 24 and 48h incubation

Dyes Isolates	DB71 (%)		DY12 (%)		AB210 (%)		RB5 (%)	
	24h	48h	24h	48h	24h	48h	24h	48h
IS1	57.50	68.63	24.93	26.46	26.30	29.40	55.06	68.46
IE1	91.46	93.03	73.73	74.83	80.83	87.73	86.13	91.80
IE2	91.90	91.20	66.63	66.63	14.63	31.50	77.73	85.76
IE3	73.33	90.30	26.96	25.26	33.96	45.73	46.36	61.03
IE4	60.90	81.70	31.16	34.23	10.00	20.83	50.00	60.00
ISEW	47.06	60.06	21.26	23.66	45.63	59.23	25.46	48.50

Table 2. Percent decolorization by pre-adapted bacterial isolates after 24 and 48h incubation

Dyes Isolates	DB71 (%)		DY12 (%)		AB210 (%)		RB5 (%)	
	24h	48h	24h	48h	24h	48h	24h	48h
IS1	70.23	88.53	46.96	50.80	33.66	40.00	60.06	71.0
IE1	93.90	94.10	92.76	92.46	87.03	88.03	91.60	92.53
IE2	87.03	91.10	51.96	59.03	41.80	49.00	78.00	82.00
IE3	92.93	93.03	47.00	48.00	27.00	31.10	64.00	75.53
IE4	74.00	80.00	35.43	45.26	28.73	31.03	66.36	74.93
ISEW	74.00	90.03	26.73	31.23	45.03	60.20	39.66	57.83

requirement of co-substrate for the dye degradation by the bacterium is stated. Many of aerobic isolates decolorize the azo compounds only in the presence of other carbon sources and therefore presumably do not use the azo dyes as carbon or energy sources. Strains of *Pseudomonas stutzeri, Acetobacter liquefaciens,* and *Klebsiella pneumoniae* were able to reductively cleave 42 - dimethylaminoazobenzene-2-carboxylic acid, Acid Red 2 (Methyl Red), during aerobic growth on NB or glucose [22,23].

There are several claims in the literature that bacteria with the ability to reduce azo dyes aerobically in a co-metabolic fashion can also use these dyes as sole source of carbon and energy [24,23].

Total six bacterial cultures were isolated, and were named as IE1 from textile industry effluent Gujrat and IE2, IE, IE4 from textile industry effluent Vasai, district Thane, IS1 from garden soil and ISEW1 from sewage sample.

Identification

All the isolates were Gram negative highly motile in nature with a morphology of cocobacilli.

On the basis of results for biochemical tests and according to bergey's manual of systematic bacteriology all the isolates were tentatively identified as IE1 (*Citrobacter freundii*), IE2*(Xenoranadus luminescence*) and IE3 (*Enterobacter cloace*) IE4 and IS1 (*Pseudomaonas* species*)* ISEW *(Enterobacter agglomerans* Aerogenic strain Biotype-G2).

Screening for the most efficient dye degraders on the basis of percentage of decolorization

Dye decolorization of four dyes AB210, DB71, DY12 and RB5 was observed by pre-adapted and non-adapted isolates IE1, IE2, IE3, IE4, IS1 and ISEW under aerobic condition. The decolorization of each dye using six isolates is shown in table:1 and 2 after 24 and 48 h respectively. It was found that percent decolorization by individual isolate was greater for DB71 dye with decolorization ranging from 66.1% to 93.9%. The second best decolorized dye was RB5 with percent decolorization of 39.6% to 91.6% whereas AB210 decolorized in the range of 27% to 87.7% and DY12 from 26.73% to 92.46%. The variation in the rate of decolorization of individual dye may be attributed to their structural difference.

Padamavathy, *et al*.[25], and Zimmermann, *et al.,* [26] reported similar observation investigating the degradability of different structures of azo dyes.

All these results revealed that DB71 was decolorized maximally by all the six isolates. Of all the isolates IE1, the isolate from textile industry effluent sample obtained from Vasai was found to be the best decolorizer as revealed from percent decolorization studies in the first set(non-adapted to dye) and the second set also (pre-adapted to dye). The second set was used to confirm the fact that whether the bacteria require prior adaptation to the dye and that the percent decolorization increases when previously adapted culture is used as inoculums. From the results it was confirmed that decolorization by pre-adapted bacterial culture did increase and the increase was significantly higher for some isolates i.e. decolorization was increased by 20% after 24 and 48 h when compared with non-adapted culture of the same bacterial isolates. According to Leena[27] effluent-adapted strains were better candidates for decolorizing the textile industry effluent than the non-adapted species.

The IE1decolorized 100mg l^{-1} dye upto 93.9% (DB 71), 92.7% of (DY12)**,** 91.6% of (RB5) and 87.7% of **(**AB210) within 24 h of incubation. The reason may be that the autochthonous or indigenous bacteria are more efficient in dye degradation as those are being evolved in the presence of the dye[28]. The evolution to alleviate the dye toxicity may be responsible for the decolorization of the dye. This is not the case with the dye decolorizing bacteria isolated form soil or other sources. This is corroborated by the fact that the sewage and soil isolates showed marked increase in dye decolorization when those were pre-exposed to the dye and then used in the dye decolorization study.

These results indicate the effectiveness of IE1 in decolorizing the four different azo dyes in short period of time (24h). It should be noted that all these dyes are sulfonated azo dyes which are normally considered to be recalcitrant than carboxylated azo dyes [29]. Sulfonated dyes are not decolorized easily, as the penetration through the cell membrane is the rate limiting step during bacterial reduction of azo dyes[30]. Such

decolorization could have occurred by oxygen insensitive azoreductase [26].

The present study has indicated that the strain IE1 can decolorize four azo dyes DB71, DY12, AB210 and RB5 even under aerobic conditions, which is in contrast to other reports of Rafii (1990)[31] and Nigam, (1996)[32], and Hu (1998)[33], according to them that azo dyes are generally resistant to attack by bacteria under aerobic conditions. It is known that azo reductase-driven bacterial decolorization of azo dyes is normally inhibited by the presence of oxygen primarily due to competition in the oxidation of reduced electron carriers like NADH with either oxygen or azo groups as electron receptor [34] but this is not true for the present work.

Secondly high effectiveness in decolorization of four sulfonated azo dyes is achieved in less time of (24 h). The decolorization of azo dyes by the IE1 is higher (Table 1 & 2) as compared to remaining five isolates. This is a new finding, somewhat similar to Kodam's study in which KMK 48 isolate showed 100% decolorization of reactive dyes one of which is sulfonated dye but here all four dyes chosen are sulfonated azo dyes.

The property like effective decolorization of direct and reactive azo dyes under aerobic condition of the microbial culture can definitely solve the problem of maintaining anaerobic condition for biodegradation of textile dye effluents.

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