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Comparison of decolorization of reactive azo dyes by microorganisms isolated from various sources

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Abstract: Azo dyes are among the oldest man-made chemicals and they are still widely used in the textile, printing and the food industries. About 10% – 15% of the total dyes used in the industry is released into the environment during the manufacturing and usage. Some dyes and some of their N-substituted aromatic bio-transformation products are toxic and/or carcinogenic and therefore these dyes are considered to be environmental pollutants and health hazards. These azo dyes are degraded by physico-chemical and biological methods. Of these, biological methods are considered to be the most economical and efficient. In this work, attempts were made to degrade these dyes aerobically. The organisms which were efficient in degrading the following azo dyes-Red RB, Remazol Red, Remazol Blue, Remazol Violet, Remazol Yellow, Golden Yellow, Remazol Orange, Remazol Black- were isolated from three different sources viz., wastewater treatment plant, paper mill effluent treatment plant and tannery wastewater treatment plant. The efficiency of azo dye degradation by mixed cultures from each source was analyzed. It was found that mixed cultures from tannery treatment plant worked efficiently in decolorizing Remazol Red, Remazol Orange, Remazol Blue and Remazol Violet, while mixed cultures from the paper mill effluent worked efficiently in decolorizing Red RB, Golden Yellow and Remazol Yellow. The mixed cultures from wastewater treatment plant efficiently decolorized Remazol Black.

Keywords: azo dyes; decolorization; aerobic transformation

Introduction

Synthetic dyes are extensively used in textile dyeing, paper printing, colour photography, pharmaceutical, food, cosmetic and other industries (Rafii, 1990). Approximately 10000 different dyes and pigments are used industrially, and over 0.7 million tons of synthetic dyes are produced annually worldwide. It is estimated that 10%—15% of dyes are lost in the effluent during such dying process (Zollinger, 1987). Major classes of synthetic dyes include azo, anthroquinone and triaryl methane dyes, and many of them are toxic or even carcinogenic compounds with long turnover times (Hartman, 1978). With the increasing use of a wide variety of dyes, pollution by dye wastewater is becoming increasingly alarming. Therefore, the discharge of highly colored synthetic dyes effluents from those industries can result in serious environmental pollution problems.

A new legislation of the European community that has restricted the use of colorants which can not be converted under any condition (Corliell, 1994). The same legislation will not be far away in India also as the pollution due to textile industries is increasing tremendously. Poor analytical methods, complexity of manufacturing and retailing chain are also contributing in environmental damages due to coloring compounds. In aquatic systems, these substances undergo various reactions. Alterations in their chemical structures can result in formation of new xenobiotic compounds which may be more or less toxic than the parental compounds. Total degradation of azo dyes is the only solution for final elimination of xenobiotics from the environment. The aerobic degradation of dyes is mostly restricted to the single dye degradation and is the main cause of failure during its application in wastewater treatment. Decolorization of azo dyes by bacteria is typically initiated by azoreductase catalyzed reduction (Zimmermann, 1982) as a consequence, conventional aerobic wastewater treatment processes usually could not efficiently remove the color of azo dyes since these compounds are often recalcitrant aerobically (Chung, 1992; Glean, 1993). But if the aerobic microorganisms are subjected to micro-aerophillic condition they are decolorizing at a faster rate (Hu, 1998). Apparently, there is still a need to develop novel biological decolorization processes leading

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to more effective clean up of azo dyes(Chung, 1992). Present studies microorganisms were isolated from various sources were compared for their degradation capability of eight dyes individually and in combination.

1 Materials and methods

1.1 Isolation of microbial culture

Mixed bacterial culture were obtained from there different sources as domestic sewage treatment plant (STP), paper mill effluent treatment plant(PMTP), tannery effluent treatment plant(TTP), by enrichment culture technique for eight dyes namely Remazol Red, Red RB, Remazol Blue, Golden Yellow, Remazol Yellow, Remazol Violet, Remazol Black, Remazol Orange, individually. Dyes were supplied by Colorchem India. The mixed culture maintained separately for individual dyes and compared for their dyes decolorization and COD reduction capability of eight dyes individually and in combination.

1.2 Media composition

Basal salt medium containing ammonium chloride, glucose(500 mg/L), peptone (200 mg/L) and yeast extract(200 mg/L) respectively along with dye(50 mg/L). Erlenmeyer flask containing 100 ml of media were inoculated with enriched microorganism isolated for individual dyes from three different sources and incubated at 30℃. Culture broth was withdrawn at interval, centrifuged, and analyzed for visible spectrum of the dyes using Shimadzu UV spectrophotometer for decolorization. The biodegradation of dyes was monitored by COD reduction during experimentation.

1.3 Synthetic wastewater preparation

The synthetic wastewater was prepared using a mixture of eight dyes. The basal salt medium was inoculated with mixture of eight dyes. The final stock has got 56 mg/L of dye solutions. The basal salt medium along with dye mixture were inoculated with microorganisms isolated from different sources.

2 Results and discussion

The concern and great attention for the treatment of industrial effluents from textile and dye manufacturing units, is steadily given throughout India. Several researchers have demonstrated the possibility of utilizing microorganisms for biotreatment of textile wastewaters(Kroiss, 1999; Park, 1996; Walker, 2000). Azo dyes are selected from the list of dyes mostly used by dye industries at Tiruppur and Karrur districts in Tamilnadu. The list of these dyes along with its λ maxima is given in Table 1. These dyes are in regularly used in textile industries and untreated wastewater is always let out into adjacent nallah.

Table 1 List of commercial dyes used in the study

Name of commercial dyes	*CI* number	No of azo bonds	λ maxima
Red RB	18055	Mono azo	520
Remazol Red	114601	Mono azo	511
Remazol Blue	20460	Mono azo	611
Remazol Black	11815	Mono azo	576
Remazol Violet	42650	Mono azo	541
Remazol Orange	60700	Mono azo	494
Golden Yellow	22910	Mono azo	412
Remazol Yellow	13065	Mono azo	418

Potential microorganisms degrading commercial azo dyes were isolated from three different sources by enrichment culture techniques. The sources were wastewater treatment plants for tannery and paper mill, as they are using dyes in their processes. The third source was sewage treatment plant. The initial concentration used in the decolorization studies was 50 mg/L. After total decolorization, 1% innoculam was suspended in fresh media and such five transfers were made and then microorganisms were suspended in fresh basal salt medium containing respective dyes. Great difficulty was experienced in isolation of stable bacterial culture biodegrading eight reactive azo dyes. The dye biodegradation without any extra carbon source was very difficult and therefore 500 mg/L of glucose was always supplemented to the media along with azo dyes. All the mixture of culture isolated from different

sources were compared for their decolorization capability and data are given in Table 2. At 50 mg/L concentration of Remazol Red and Red RB were decolorized efficiently within 4 h by microbial of culture isolated from STP and PMTP. Remazol Blue was decolorized only by microorganisms from STP and TTP, but not by PMPT. Remazol Black was decolorized by microorganisms from STP and not by other two sources. PMTP microorganisms were efficient in decolorization of Remazol Voilet than STP and TTP. Remazol Orange and Remazol Yellow were efficiently decolorized by the microorganisms isolated from all the three sources. Golden Yellow was very difficult to decolorized and only 42%—57% decolorization was achieved by the microorganisms isolated from all the three sources. Similar decolorization trend was observed for higher concentration also. The most of the dyes at 100 mg/L were decolorized by the microorganisms isolated from all the sources except for the Red RB, which was decolorized at still higher concentration also (200 mg/L). The COD removal during the decolorization is given in Table 3 and COD removal is also varies for with the source of isolation. The final pH of the culture broth increased from 7.0 to 8.6. At the same time ammonia concentration in the broth was also increased indicating cleavage of the azo bond(data not shown).

Table 2 Microorganisms isolated from different sources for azo dyes degradation

Name of commercial dyes	Concentration, mg/L	Sources of isolation, decolorization, %		
		Sewage treatment plant	Tannery wastewater treatment	Paper mill plant waste treating lagoon
Remazol Red	50	80.9	95.38	86.36
	75	76.5	90.00	83.30
	100	73.3	86.60	80.60
Red RB	50	98.1	89.40	87.09
	100	93.35	84.51	85.60
	200	85.5	82.81	85.20
Remazol Blue	50	69.23	69.23	25.00
	75	85.00	41.60	21.10
	100	85.18	ND	ND
Remazol Black	50	75.00	20.00	14.28
	100	33.30	19.25	5.50
Remazol Violet	50	77.27	94.40	80.00
	100	73.60	63.15	68.42
Remazol Orange	50	81.10	86.60	82.80
	100	56.52	80.00	60.00
Golden Yellow	50	57.14	55.50	42.42
	75	25.00	36.60	17.31
Remazol Yellow	50	73.17	75.40	61.53
	100	23.60	24.32	50.00

Decolorization of dyes from synthetic effluents: The dye content is an industrial azo waste stream typically varies from 10 – 50 mg/L. However changes in operating conditions do occur and it is important to know that azo dyes decolorizing microorganisms can handle higher concentrations. Therefore a comparison was made for all the microorganisms isolated from all the three sources for a synthetic wastewater containing all the eight azo dyes and results are given in Table 4. Under normal condition all the three able to decolorize synthetic wastewater within 24 h, but COD removal was more by TTP microorganisms than STP and PMTP. The degree of the dye decolorization for the wastewater was measured spectrophotometrically in the supernatant of the culture grown in synthetic wastewaters.

Table 3 COD removal and ammonia formation during the biodegradation of azo dyes by mixture of culture

Dyes	Conc., mg/L	Sewage treatment plant isolates COD removal, %	Tannery wastewater treatment plant isolates COD removal, %	Paper mill waste treating lagoon isolates COD removal, %
Red RB	50	90.87	81.80	89.30
Remazol Red	50	75.78	68.56	78.74
Remazol Blue	50	82.06	69.60	72.60
Remazol Black	50	65.33	87.20	85.96
Remazol Violet	50	28.30	25.85	35.10
Remazol Orange	50	91.15	87.33	85.76
Golden Yellow	50	89.73	83.30	95.16
Remazol Yellow	50	65.95	81.95	69.36

Table 4 Biodegradation of mixed azo dyes using microorganisms isolated from different sources

Eight dyes mixture, mg/L	Sewage treatment plant(STP) COD, mg/L			
	0	24	48	96
56	913.92	473.28	450.00	—
116	962.88	669.12	669.12	603.84
232	1041.5	350.08	350.08	340.00
288	1147.4	293.76	255.12	1126.1
Tannery treatment plant(TTP) COD, mg/L				
56	913.92	48.96	47.00	—
116	995.52	179.52	179.52	97.92
232	1083.6	261.12	211.12	210.08
288	1126.1	408.00	374.17	374.17
Paper mill effluent treatment plant(PMTP) COD, mg/L				
56	913.92	261.12	230.12	—
116	946.56	326.40	261.12	228.48
232	1062.4	300.00	300.00	300.00
228	1104.89	261.12	238.11	238.11

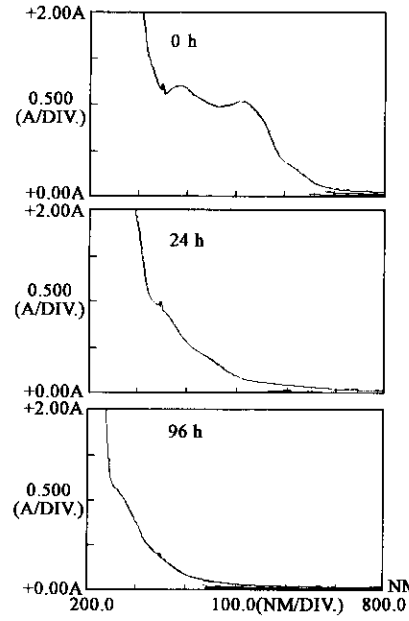

Fig. 1 Absorption spectra for synthetic wastewaters

Fig. 1 shows a scan for the wastewater containing a mixture of dyes at initial concentration and decolorization pattern during ninety six hours incubation for microorganisms isolated from TTP. The extent of decolorization was with the same efficiency in dyes mixture sample as compared to individual dyes decolorization (Table 2). All then dyes individually as well as in the mixture were completely decolorized within 24 h. At higher dyes concentrations the microorganisms becomes progressively less capable of eliminating dyes from the synthetic wastewaters. The values obtained for bio-kinetic constants for synthetic wastewater are presented in Table 5, which indicates that the average values of μ increase with increase in concentration till 100 mg/L, but decline at higher concentration. Evaluation of bio-kinetic constant for synthetic wastewater have shown that the maximum specific growth rate for STP microbial culture is more than other two sources. The initial step in biodegradation of azo dyes is a reduction cleavage of the azo group. An enzyme azoreductase is considered to be responsible for the cleavage of azo bond (– N = N –). The enzyme azoreductase is secreted by the cells when there is lack of oxygen and in turn decolorization is achieved, but yield in poor growth of the cells. The enzyme azoreductase is considered to be sensitive to oxygen(Wuhrmann, 1980). However, a number of relatively simple azo compounds and dyes have been transferred under aerobic conditions by specific bacterial strains. Two such examples include the azo dyes reduction by *Bacillus* sp. *OV*1 – 2(Sugiura, 1999) and the reactive cleavage of azo bond of orange Ⅱ by *Pseudomonas* sp. strain KF46(Zimmermann, 1982). Yatome *et al*. (Yatome, 1991) reported that the permeability to the dye and presence of azoreductase affected the reduction of azo dye by pure culture of *P. stutzeri*, *B. subtilis* and *P. cepacia* under aerobic condition. They concluded that the microbial reduction

Table 5 Kinetics constant determination for synthetic wastewater containing mixture of eight azo dyes under batch condition

Azo dyes concentration in synthetic wastewater, mg/L	μ, mg/h		
	Sewage treatment plant(STP) treatment	Paper mill effluent plant(TTP)	Tannery treatment plant(PMTP)
56	0.0055	0.0055	0.0055
116	0.0052	0.0060	0.0056
232	0.0045	0.0055	0.0045
288	0.0031	0.0050	0.0017
	μ_{max}, mg/h		
	0.00807	0.00439	0.00313

of azo dyes under aerobic conditions was microorganism and dye specific. Therefore, azo dyes reduction is typically not achieved by unassimilated bacterial consortia under the aerobic conditions of activated sludge process. The rate of decolorization of individual dye varied. This may be attributed to their structural difference. Zimmermann *et al*. (Zimmermann, 1982) reported similar observation investigating the degradability of different structures of azo dyes. Azo compounds with an hydroxy or amino groups are more likely to be degraded than those with a methoxy, sulpho or nitro group(Pagga, 1986; Shaul, 1991). The advantages of mixed culture is apparent as some strains can collectively carry out biodegradation tasks that no individual pure strain can achieve. Presence of supplementary carbon source for effective dye decolorization and biodegradation was necessary. Under batch experimentation condition the azo dye decolorization efficiency and COD removal varied with the individual dye. But in synthetic wastewater such preferential removal did not observed as always decolorization was achieved within 24 hours. Most of the studies on pure and mixed cultures of microorganisms degradation is restricted to substrate specificity of the azoreductase, with the changes in the location or the type of substituents on the aromatic rings typically reducing enzyme activity(Zimmermann, 1982). Since cleavage of the azo bond by an azoreductase is invariably the first step in the biotransformation of azo dyes, organisms capable of opening aromatic ring or removal of functional groups can they lead to decolorization. The further thinking in concept of aerobic microbial decolorization and structure specificity of azoreductase is very much questionable and these approaches are currently being investigated.

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