Aerobic Decolorization of Reactive Azo Dyes in Presence of Various Cosubstrates

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Azo dyes belong to the most important group of synthetic colorants. They are generally considered as xenobiotic compounds which are very recalcitrant to biodegradation. Azo dyes are deficient in carbon content and therefore decolorization is possible only under co-metabolic condition. In this work, attempts were made to degrade these dyes aerobically. The organisms which were efficient in degrading the azo dyes-Red RB, Remazol Red, Remazol Blue, Remazol Violet, Remazol Yellow, Golden Yellow, Remazol Orange, Remazol Black, were isolated from three different sources and were mixed to get consortia. The efficiency of azo dye degradation by consortia was analyzed in presence of various cosubstrates, such as glucose, starch, lactose, sewage and whey water, and their effects on dye decolorization have been studied. It was observed that starch was the best source of carbon for decolorization of reactive azo dyes. In presence of 250 mg l^{-1} of starch, all the reactive dyes decolorized within 24 hours with the reduction in COD in the range of 75.15–95.9 %. The COD reduction was the maximum in Remazol Orange and Red RB followed by Remazol Yellow, Remazol Violet, Remazol Black, Remazol Red, Golden Yellow and Remazol Blue. The decolorization of above referred dyes in presence of different wastewaters such as sewage and whey waste, showed that whey is the better cosubstrate for aerobic decolorization.

Keywords:
Decolorization, Aerobic biodegradation, Azo dyes, wastewater

Synthetic dyes are extensively used in textile dyeing, paper printing, color photography, pharmaceutical, food, cosmetics and other industries.[1] Approximately 10,000 different dyes and pigments are used industrially, and over 0.7 million tons of synthetic dyes are produced annually, worldwide. In 1991, the world production of dyes was estimated 668,000[2] of which azo dyes contributed 70 %[3]. During dying process, a substantial amount of azo dye is lost in a wastewater[2]. *Zollinger* has reported 10–15 % of dyes are lost in the effluent during dying process.[4] Major classes of synthetic dyes include azo, anthraquinone and triaryl methane dyes, and many of them are toxic or even carcinogenic compounds with long turnover times.[5] With the increased use of a wide variety of dyes, pollution by dye wastewater is becoming increasingly alarming. Therefore, the discharge of highly colored synthetic dye effluents from those industries can result in serious environmental damages. Besides color is the first contaminant recognized in textile wastewater and has to be removed before discharging into receiving water body.

A new legislation of the European community has restricted the use of colorants which can not be converted under any condition.[6] The German government, through the forth amendment to the consumer goods ordinance, has recently banned the imports of textiles, leather, and other item, dyed with azo dyes, based on carcinogenic amines (MEF, New Delhi Notification). The same legislation will not be far away in India, as the pollution due to textile industries is also increasing tremendously. 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[7] 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.[8,9] But if the aerobic microorganisms are subjected to micro-aerophillic condition they are decolorizing at a faster rate.[10] Apparently, there exist a need to develop novel biological decolorization processes leading to more effective clean up of azo dyes.[8] In the present studies efforts

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have been made to decolorize reactive azo dyes and simulated wastewater in presence of different co-metabolites using microbial consortia.

Materials and methods

Isolation of microbial culture Mixed bacterial culture were obtained from the 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 details, which are given in Table 1. Dyes were supplied by Color-Chem India. The microbial consortia was maintained separately for individual dyes and in combination.

Table 1 – *List of commercial azo dyes used in the study*

Name of commercial azo dyes	CI Number	No of azo bond	λ_{max}/nm	γ_{COD}/mg l^{-1} *
Red RB	18055	Mono azo	520	144.0
Remazol Red	114601	Mono azo	511	147.6
Remazol Blue	20460	Mono azo	611	169.2
Remazol Black	11815	Mono azo	576	162.0
Remazol Violet	42650	Mono azo	541	90.0
Remazol Orange	60700	Mono azo	494	54.0
Golden Yellow	22910	Mono azo	412	108.0
Remazol Yellow	13065	Mono azo	418	90.0

*Dye mass concentration is 50 mg l^{-1}

Media composition Basal salt medium, made from phosphate buffer (1 mol l^{-1}) pH 7.0, was used along with yeast extract (200 mg l^{-1}) and dye (50 mg l^{-1}) in the studies. Experiments were performed in flask containing 100 ml of media inoculated with enriched microbial consortia and incubated at 30 °C. After total decolorization, 1 % inoculate was suspended in fresh media and such five transfers were made, and then microorganisms were suspended in fresh basal salt medium containing respective dyes. Culture broth was withdrawn at intervals, centrifuged, and analyzed for UV-visible spectrum of the dyes, using Shimadzu UV spectrophotometer (160A) for decolorization. The biodegradation of dyes was monitored by reduction in chemical oxygen demand (COD) during experimentation.[11]

Simulated wastewater preparation The simulated wastewater was prepared using a mixture of eight dyes. All the dyes were mixed together to get stock solution of mixture of dyes 5600 mg l^{-1} (700 mg l^{-1} of each dye). The stock solution was supplemented to basal salt medium to get final concentration of 56 mg l^{-1}. The basal salt medium along with dye mixture was inoculated with microbial consortia.

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 wastewater.[12–14] Azo dyes are selected from the list of dyes mostly used by dye industries at Tiruppur and Karur districts in Tamilnadu, India. These dyes are regularly used in textile industries and untreated wastewater is always let out into adjacent channels. In India, the most of textile units are scattered and operated from houses, therefore, it is necessary to collect and treat the waste in common effluent treatment plant. Biological methods are simple to use and low cost is involved in operation.[15] Potential microorganisms identified as *Pseudomonas sps*, *Bacillus sps Halomonas sps, Orthrobacter sps, Micrococci sps* degrading commercial azo dyes, were mixed to form consortia. The dyes are deficient in carbon content and biodegradation without any extra carbon source was very difficult. Therefore, different co-substrates such as glucose, starch, lactose (250 mg l^{-1} each), were supplemented in the medium and decolorization of all the eight dyes were studied individually (Table 2). Red RB, Remazol Red were efficiently decolorized (91–94 %) in presence of glucose as compared to other dyes. But reduction in COD was only 62–66 % (Figure 1). Starch, as cometabolite, gave constant decolorization (75–95.6 %) except for Remazol Black. During decolorization in presence of starch, as co-metabolite, COD removal was also fairly constant (80–95.11 %). When the comparison was made for lactose, both decolorization potential and COD reduction, was found to be less. The initial COD removal was 36.68 – 74.84 % except for Remazol Orange (5.85 %), Remazol Blue (13.0 %) and Remazol Yellow (21.9 %). *Nigam* et.al[16] have also observed that the medium supplemented with glucose, glycerol and lactose, have given better degradation of dyes. *Hu*[14] in his studies observed that the color removal ability for several reactive dyes

Table 2 – *Decolorization of azo dyes in presence of co-substrates*

Azo Dyes	Decolorization, %				
	Glucose	Starch	Lactose	Sewage	Whey water
Red RB	94.70	86.60	95.00	80.00	86.60
Remazol Red	91.20	95.60	70.10	65.00	84.50
Remazol Blue	73.00	91.60	45.20	80.30	78.57
Remazol Black	80.00	50.00	48.90	68.00	42.85
Remazol Violet	80.10	80.00	70.00	58.00	37.50
Remazol Orange	78.20	85.70	45.20	85.00	83.30
Golden Yellow	56.80	75.00	55.00	70.00	77.70
Remazol Yellow	78.40	84.80	60.10	58.00	84.12

under low carbon source (1.25 g l^{-1}). But the concentration of co-substrate in present study was just enough to support initial growth. The decolorization and COD removal did not go together. When all the co-substrates were compared, starch seemed to be better co-substrate, both, with respect to decolorization as well as for biodegradation.

Decolorization of all the dyes in presence of sewage and whey water as co-substrates, were also compared in order to replace co-substrate by industrial wastewater, and results are given in Table 2 for decolorization, and Figure 1 for COD reduction. In presence of sewage 65 –85 % decolorization was obtained for eight dyes except for Remazol Violet (58.0 %) and Remazol Yellow (58.00 %). The initial COD removal was 61.53–88.75 %. In presence of whey water 77.70 –86.60 % decolorization was obtained for eight dyes except for Remazol Violet (37.5 %) and Remazol Blue (42.85 %). The initial COD removal was 62.50 – 87.90 %. Even though lactose was poor as co-substrate for decolorization of dyes, whey water had shown better reduction in COD and decolorization than sewage. This may be due to milk associated proteinaceous matter supporting decolorization. *Knapp* and *Newby*[17] have also reported that decolorization is favored by highly proteinaceous media.

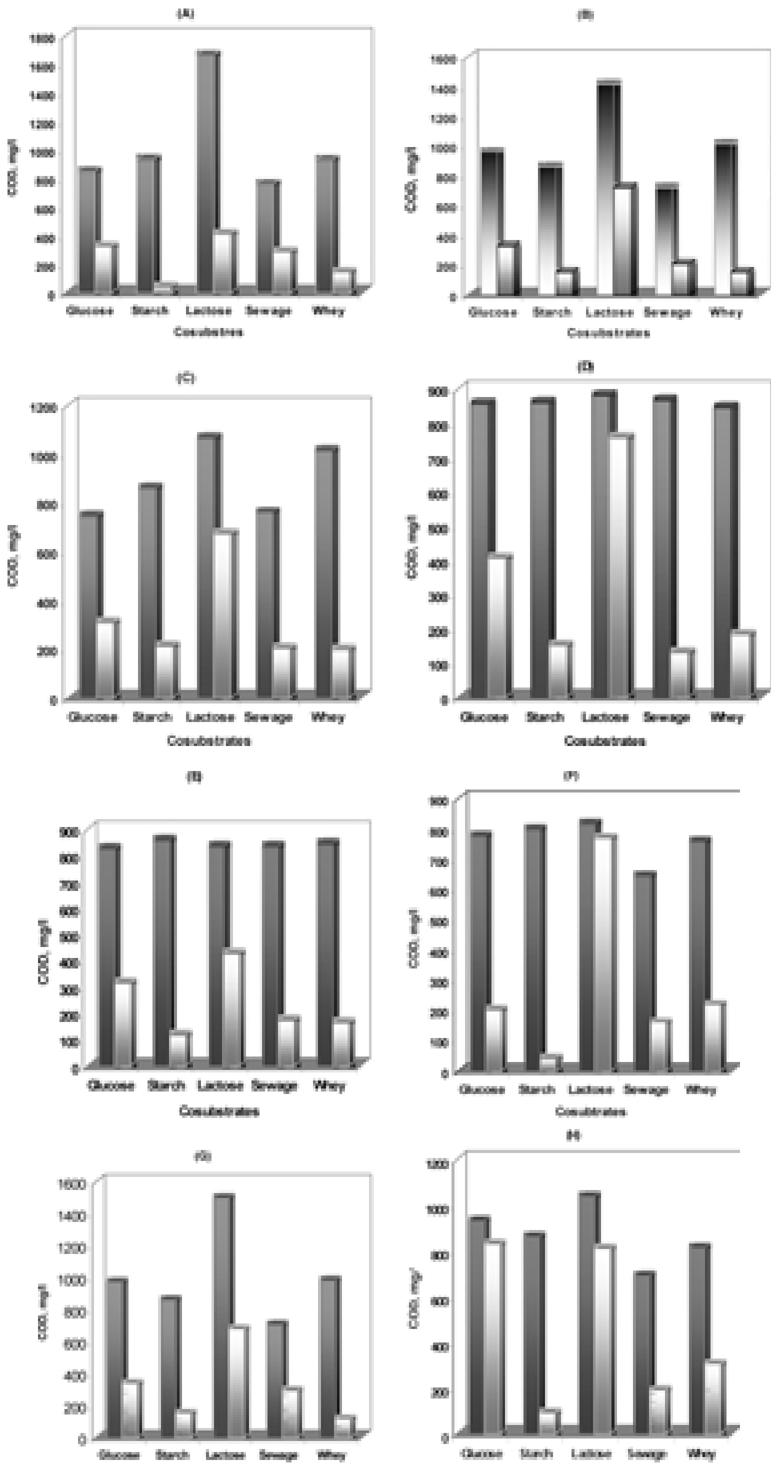
Decolorization of dyes from simulated wastewater

The dye mass concentration is an industrial azo waste stream typically varies from 10–50 mg l^{-1}.[18] 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 carbon sources for a simulated wastewater containing all the eight azo dyes and results were given in Table 3. Under normal condition all the three are able to decolorize simulated wastewater within 24 h, but COD removal was more efficient in presence of whey water followed by starch, glucose and sewage. Lactose was very poor metabolite for simulated wastewater decolorization. The extent of decolorization was with the same efficiency in dyes mixture sample as compared to individual dyes decolorization. All dyes, individually as well as in the mixture, were ther completely decolorized within 24 hours. An enzyme azoreductase is considered to be responsible for the cleavage of azo bond (-N = N-). *Yatome* et al[19] 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. The rate of decolorization of individual dye varied. This may be attributed to their structural difference. *Zimmermann* et al[7] reported similar observation investigating the degradability of different structures of azo dyes. Under batch experimentation condition the azo dye decolorization efficiency and COD removal varied with the individual dye. But in synthetic wastewater such preferential removal was not observed as decolorization was always achieved

Table 3 – *Azo dyes biodegradation from synthetic wastewater under aerobic co-metabolic conditions*

Various carbon co-metabolites	Initial concentration of dyes in synthetic wastewater mg l^{-1}	γ_{COD}, mg l^{-1}			Decolorization %
		Initial	Final	Removal %	
Glucose	112	913.92	48.96	94.85	94.60
Lactose	112	1068.3	274.68	74.28	95.00
Starch	112	1078.4	223.18	79.30	97.00
Sewage	112				92.00
Whey water	112	1071.6	135.68	86.60	95.00

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 substituent on the aromatic rings typically reducing enzyme activity[7]. 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 lead to decolorization. The further thinking of concept of aerobic microbial decolorization and structure specificity of azoreductase, is very much questionable and these approaches are currently being investigated.



F i g . 1 – *Microbial reduction of azo dyes in presence of cosubtrates Red RB (A), Remazol Red (B), Remazol Blue (C), Remazol Black (D), Remazol Voilet (E), Remazol Orange (F) and Golden Yellow (G), Remazol Yellow (H)*

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