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BACTERIAL DECOLORIZATION OF ACID YELLOW DYE OBTAINED FROM TEXTILE INDUSTRY EFFLUENTS

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ABSTRACT

Dyes are threat to aquatic as well as human life. These are often introduced in the water bodies from textile or paper industries with improper treatment. Chemical treatments for dye decolorization are quite costly; another disadvantage is decolorization of these dyes is not complete. The bacterial flora in the effluent of textile industries is continuously exposed to dyes. Few of this might be excellent dye decolorizers and also dyes are used as source of carbon and nitrogen by few bacteria. The bacterial decolorization of dyes is preferable one because the dyes are completely decolorized by these bacteria. The case study was carried by selecting acid yellow dye. The efficient dye decolorizing bacteria includes *Pseudomonas* sp*, Alteromonas* sp*, Enterococcus* sp*, Serratia* sp and *Enterobacter* sp. Periodic studies on utilization of these dyes are carried out. Dye decolorization was at peak level towards the end of second day. Hence this also suggest when a suitable strategy is developed for the decolorization of dyes two day period will be sufficient to treat the effluent by these bacteria.

KEYWORDS: bacteria, dye, decolorization, periodic study.

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INTRODUCTION

The scarcity of natural dyes has promoted the use of synthetic dyes. These synthetic dyes are selected on the basis of resistance to washing and attack by microorganisms. Stronger the dye resist to microbial attack makes it more valuable. The stronger and intense synthetic dyes which are not degraded by bacteria emerged as xenobiotics. A large quantity of these dyes is thrown in the water bodies without any special care. These dyes cause respiratory disorders as well as skin and lung cancer[1]. One cannot ignore the indirect and excessive use of azo dyes in daily routine. These are used as coloring agents in food, cosmetics, laboratory staining processes, leather, plastic, toys industries, house paints and much more[2]. More than 2000 azo dyes are produced annually constituting 50% of total dyes. Azo dyes are the most popular synthetic dyes and are resistant to microbial attack as well. The annual production of these dyes is more than $7x10^5$ tones. Another disadvantage of synthetic dye production is the consumption of large volumes of water at almost every step. It is also important to note that 10% of the dye does not bind to clothes which ultimately are discharged in the water bodies[3]. Azo dyes are classified into three major groups such as acid, basic and direct dyes[4]. Immediate effect of these dyes is on aquatic plants[5]. The high color content of these dyes ultimately affects photosynthetic activity of plants and cyanobacteria. These further causes foul smelling of water reservoirs. The continuous production of dyes and continual exposure of human beings is great concern for increased cases of cancer in developing countries[6]. A dual system was adopted for removal of dyes; one was mechanical and other was biological. The decolorization of the dye employing rice hull was 89.71% and by *Schizophyllum* sp was 60.44% still the biological method was proved feasible[7]. Other non-biological method includes mahogany sawdust and rice husk. These were found effective for the removal of dyes quite economically[8]. Fly ash was also attempted for removal of dyes[9]. As reviewed by Robinson et al., (2001) physicochemical methods has double disadvantage in addition to the expensive nature of these methods these also create a disposal problem[10].

Biological methods of dye degradation are dominant being able to degrade dye rather than deposition. Aerobic bacteria exceeds in utilizing the azo dyes as sole source of carbon and nitrogen[11]. Energy as sunlight and carbon source as dye is definitely an economical and feasible process. *Rhodopseudomonas palustris* a photoheterotrophic bacterium was isolated from Lake Akkaya by Celik et al.,[12]. The fastest decolorization of reactive blue R250 (100mg/L) was achieved within 8 hours. The bacterium was characterized as *Pseudomonas otitidis*[13]. Engineered cells were introduced for the first time to decolorize dyes. Wei et al, (2012) studied the laccase activity from engineered *P. putida* cells[14]. Decolorization of crystal violet was studied by Thorat and Sayyad[15]. Around seven cultures were studied for decolorization of crystal violet with nearly 92% efficiency. Moderate temperature and pH were found sufficient for decolorization; it was also revealed that addition of simple organic substrate like glucose did not have any marked influence on decolorization activity. Fungi like *Polyporus rubidus* was undertaken by Dayaram and Dasgupta[16]. In this study we have obtained dye degrading bacteria from textile industry effluent near Amravati district (Maharashtra). Characterization and efficiency of these bacteria for dye degradation is studied. The periodic studies on dye degradation have also been carried.

MATERIALS AND METHODS

(i) Enrichment of bacteria

The industrial effluents were collected from the textile industry in Amravati district. For enrichment of culture heterogeneous population was first grown aerobically in a medium containing minimal salt media and 100mg/L dye followed by incubation at 37°C for 24 hrs. The medium contained(g/l) KCl- 0.1, KH_2PO_4-3.0,

K_2HPO_4-7.0, $CaCl_2$-0.01, $MgSO_4.7H_2O$- 0.5, $FeSO_4.7H_2O$- 0.116, H_3BO_3- 0.232, $CoCl_2.6H_2O$- 0.41, $CuSO_4.5H_2O$- 0.008, $MnSO_4.H_2O$- 0.008, $ZnSO_4$- 0.174 and pH7 which was supplemented with acid yellow dye 0.1%, yeast extract 0.05% and glucose 0.25%. Colonies appeared were repeatedly subcultured to obtain pure cultures.

(ii) Carbon source utilization by the isolates

Pure cultures were designated M1 to M12. After repeated screening twelve isolates were obtained which were further chosen for morphological and biochemical characterization.

(iii) Analysis of Decolorization

A standard graph was prepared and dye concentration before and after incubation was determined. However the method of percent dyes decolorization is popular hence this method was considered. Dye decolorization was determined by following formula.

$$\text{Decolorization \%} = \frac{\text{Initial O.D.- Final O.D.}}{\text{Initial O.D.}}$$

RESULTS AND DISCUSSION

(i) Enrichment of bacteria

Cultures were enriched in above mentioned media. The faster decolorization of the broth was noted and these culture flasks were selected for pure culture studies on agarized media. Twenty culture flasks were studied for decolorizations of the acid yellow dye through which twelve culture flask on the basis of dye decolorization were selected for further studies.

(ii) Carbon source utilization by the isolates

After enrichment of bacteria in acid yellow dye containing media; the isolates were undertaken for morphological and biochemical studies. On the basis of these characterization representatives of *Enterobacter* sp*, Acinetobacter* sp*, Pseudomonas* sp*, Alteromonas* sp*, Enterococcus* sp*, Serratia* sp and *Staphylococcus* sp are reported. These bacteria are further undertaken for acid yellow dye decolorization efficiency (Table 1).

Table1

Morphological properties and sugar utilization by various isolates

Culture designation	Cultural characteristics							Morphological properties				Biochemical characteristics																	Identified Bacterial species
	Color	Size	Shape	Margin	Elevation	Opacity	Consistency	Gram reaction	Shape	Endospore	Motility	Catalase	Oxidase	Indol	M R	VP	Citrate	Arabinose	Glucose	Fructose	Lactose	Mannitol	Maltose	xylulose	Sucrose	Salicin	Rhamnose	cellobiose	
M1	Colorless	0.1mm	Circular	Entire	Convex	Opaque	Soft	-	Rod	N E	M	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	*Enterobacter sp*
M2	Colorless	0.15mm	Circular	Entire	Convex	Opaque	Soft	-	Rod	N E	NM	+	-	-	-	-	+	+	+	+	+	+	+	+	-	-	+	+	*Acinetobacter sp*
M3	Colorless	0.1mm	Circular	Lobate	Convex	Opaque	Soft	-	Rod	N E	M	+	-	-	-	-	+	+	+	+	+	+	+	+	-	-	+	+	*Enterobacter sp*

M4	Colorless	0.5mm	Circular	Entire	Convex	Translucent	Soft	-	Rod	N E	NM	+	-	-	-	-	+	+	+	+	-	+	+	+	-	-	+	+	*Acinetobacter sp*
M5	Colorless	0.1mm	Circular	Entire	Convex	Translucent	Hard	-	Rod	NE	NM	+	-	-	-	-	+	+	+	+	+	+	+	+	-	-	+	+	*Acinetobacter sp*
M6	Green	0.5mm	Circular	Entire	Convex	Opaque	Soft	-	SR	NE	M	+	+	-	-	-	+	-	+	-	-	-	-	-	-	+	-	-	*Pseudomonas sp*
M7	Colorless	0.5mm	Circular	Entire	Convex	Translucent	Soft	-	Rod	NE	M	+	-	-	-	-	+	+	+	+	-	+	+	+	-	-	+	+	*Alteromonas sp*
M8	Colorless	0.5mm	Circular	Entire	Convex	Opaque	Soft	+	Cocci	NE	M	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	*Enterococcus sp*
M9	Colorless	0.5mm	Circular	Entire	Convex	Opaque	Hard	-	SR	NE	M	+	-	-	-	-	+	+	+	+	-	+	+	+	-	-	-	+	*Serratia sp*
M10	Creamish	0.5mm	Circular	Entire	Convex	Opaque	Hard	+	Cocci	NE	NM	+	-	-	+	-	-	-	+	+	-	+	+	-	+	+	-	-	*Staphylococcus sp*
M11	Colorless	0.1mm	Circular	Entire	Convex	Opaque	Soft	-	Rod	NE	M	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	*Enterobacter sp*
M12	Colorless	0.1mm	Circular	Entire	Convex	Opaque	Soft	-	Rod	NE	M	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	*Enterobacter sp*

SR- Short rod, NM-Nonmotile, NE-non-endospore forming

(iii) Analysis of Decolorization

Graph1

Percent decolorization by various isolates during two day period

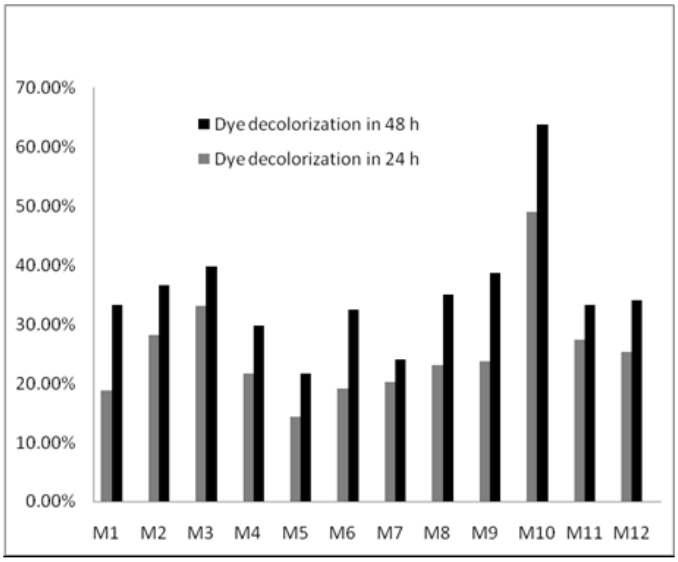

Table2
Decolorization percent of various isolates

Sr. no.	Isolates	OD at 580 nm	Percent decolorization after 24 h	OD at 580 nm	Percent decolorization after 48 h
M1	*Enterobacter* sp.	0.466	18.81%	0.383	33.27%
M2	*Acinetobacter* sp.	0.412	28.22%	0.364	36.58%
M3	*Enterobacter* sp.	0.384	33.10%	0.346	39.72%
M4	*Acinetobacter* sp.	0.450	21.60%	0.403	29.79%
M5	*Acinetobacter* sp.	0.492	14.28%	0.450	21.60%
M6	*Pseudomonas* sp	0.464	19.16%	0.388	32.40%
M7	*Alteromonas* sp.	0.458	20.20%	0.436	24.04%
M8	*Enterococcus* sp.	0.442	22.99%	0.373	35.01%
M9	*Serratia* sp.	0.438	23.69%	0.352	38.67%
M10	*Staphylococcus* sp.	0.293	48.95%	0.208	63.76%
M11	*Enterobacter* sp.	0.417	27.35%	0.383	33.27%
M12	*Enterobacter* sp.	0.429	25.26%	0.379	33.97%
Initial optical density was 0.574.					

Very less dye was decolorized during first day. This might have been because of insufficient growth of bacteria but during second day it was at the peak. Maximum dye decolorization was by *Staphylococcus* sp i.e. around 49%. The lowest was decolorized by *Acinetobacter* sp (M5) around 14%. Considerable enhancement was observed after second day where maximum dye was decolorized by *Staphylococcus* sp i.e. 63.76%. Followed by *Staphylococcus* sp other bacteria were *Enterobacter* sp (M3), *Serratia* sp (M9), *Acinetobacter* sp.(M2), *Enterobacter* sp.(M12), *Enterococcus* sp.(M8), *Enterobacter* sp (M1), *Enterobacter* sp (M11), *Pseudomonas* sp (M6), *Acinetobacter* sp.(M4), *Alteromonas* sp(M7) and *Acinetobacter* sp (M5). Unlike other dye decolorization studies dye decolorization was not achieved more than 90% rather it was less than 65% only (Table 2 and graph 1). Species of *Enterobacter*, *Pseudomonas* and *Morganella* were reported to degrade acid orange. In our study we have extracted these bacteria to decolorize acid yellow dye. Various aspects were taken into consideration for degradation of azo dyes by Barragan et al, (2007). One of the aspects was degradation of dye on the surface of solid media. It is important for any degradation the cell surface area should be more in contact with the substrate which is achieved by solid media[17]. *Acinetobacter calcoaceticus* was found to degrade 20 azo dyes. About 91% of dye was removed under anaerobic condition whereas aerobically very less dye was removed[18]. Similar results were also observed in case of *Proteus mirabilis*. Species of *Serratia marcescens* were also found to degrade direct orange[19]. In this study similar organism was shown to degrade acid yellow dye. The dye is quite often used in textile industries. Genus variation was also observed in case of *Enterobacter*, and *Acinetobacter*. Dye decolorization was studied during three day period where it was observed that maximum decolorization was towards end of second day. Marginal difference was observed in third day decolorization. Hence two day period would be sufficient to degrade acid yellow dye using these micro-organisms.

CONCLUSION

There are various factors which affect the efficiency of dye decolorization of which environmental factors are major where these bacteria are adapted. Therefore efficient dye

degrader from other environment might be ineffective. The dye degrading bacteria isolated from this industrial effluent may considered suitable for treatment of the same. However the most efficient dye degrader could decolorize only 63.76% of acid yellow dye. Further these dye decolorizing bacteria can be employed for removal and / degradation of dyes into non-toxic compounds.

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