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Study on the decolorization of dyes by microorganisms

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Abstract—Thirteen strains of bacteria with ability of dye decolorization were isolated from activated sludge and biofilm collected from textile wastewater treatment plants, and identified as *Alteromonas, Klebsiella, Pseudomonas, Enterobacter. Alcaligenes* and *Corynobacterium*. These strains D_{33}, D_{32}, D_4 of *Alteromonas* can decolorize 29 kinds of dyes with good decolorizing activities of about 2.6 mg dye/gram wet cells.h^{-1}. The optimum pH was 7–8 and the temperature was 37°C. The growth of bacteria was 3 times better in shaking cultures than in static cultures. But the decolorization rate of dyes was 4–9 times more in the latter than in the former. The results from the examination of the reaction products of Diamond Chrome Red B and Diamond Narry Blue RRN indicated the possibility of biodegradation of these dyes by *Enterobacter* D_{17}.

Keywords: dyes; microbial decolorization; microorganism.

INTRODUCTION

Dyes are main pollutants in the textile and dyeing wastewater. The studies on the decolorization and degradation of dye have been conducted by *Psuedomonas Sphaetotilus natuna, Arthrobacter, Oxidative Red Yeast, Bacillus Subuilis* (Chizoko, 1981; Horitsu, 1977; Kuhrmann, 1985; Kuhrmann, 1980). The Azo dyes are commonly used as dyes to be tested. The research work on this field has just started (Peng, 1984; Zhong, 1985) in China. It is necessary to carry out research widely on the increasing sorts of dyes to be tested. We have begun upon the research on microbial degradation and decolorization of dyes. According to the characteristics of textile and dyeing wastewater in our country, we have isolated the strains of bacteria with high activities in the decolorization and degradation of dyes, and investigated the conditions and mechanism of degradation of dyes.

MATERIAL AND METHODS

Isolation of decolorizing bacteria

The composition of medium for isolating bacteria was as follows (g/L): KH_2PO_4, 1.8; Na_2HPO_4. 12 H_2O, 3.5; $MgSO_4$, 0.2; $FeCl_3$, 6; H_2O, 0.01; peptone, 2.0; beef extract, 3.0; glucose, 5; dye, 0.05. Sterilization for 30 min under 3.6 kg/cm^2 of steam pressure.

Samples for enrichment cultures

8 samples were collected from biofilms and activated sludges from textile and dyeing wastewater treatment plants.

The samples were inoculated separately in 50 ml medium containing various dyes such as Carmoisine B, 2GL, Mordant Brown RH, Mordant Blue B, Mordant Yellow GG and then were incubated in static at 28°C for 24h. Activity of every strain of bacteria in decolorizing dye was determined. Some single colonies were picked out from the plate cultures. The strains of bacteria which are able to decolorize various dyes at high speed and efficiency were selected and identified.

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Identification of bacteria

The strains of bacteria isolated were identified according to Common Method for Identification of General Bacteria (Bacterial Classification Group of Institute of Microbiology, 1978) and Bergey's Manual of Systematic Bacteriology (Krieg, 1984).

Measurement of decolorizing activities

Visual observation was used to select decolorization bacteria.

The decolorizing activities of selected bacteria were measured with Model 751 Spectrophotometer. The reaction of dye decolorization was carried out at 37°C under static condition. The suspension was centrifugated for 20 min at 8000 r/min to remove the cells. Absorption of supernatant was measured in the visible portion of spectrum and the reaction supernatant without inoculation was taken as control. The rate of decolorization was calculated.

Sources of dyes

In this study water-soluble dye shown in Table 1 were used. They belong to Azo dyes, Thiphenylmethane dyes and Anthraquinone dyes.

Table 1 Tested dyes

Types of dyes	Names of dyes
Acid mordant dyes	Carmoisine B 2GL, Mordant Brown RH, Acid Blue GGN, Mordant Blue B, Mordant Red B, Chrome Crey B, Mordant Red-sw, Mordant Orange G, Diamond Narry Blue RRN, Chrome Black T, Mordant Red s-80, Mordant Brown MM, Mordant Yellow GG,
Active mordant dyes	Cibacron Brilliant Red K_2B, Procion Violet K-3R, Procion Brown KB-3R, Procion Blue KGL, Keamira Brilliant orange KN-4R, Keamira Brilliant K2GV, Procion Brilliant Blue X-3R, Procion Blue KN, Mikacion Brilliant Red 5BS, Procion Black KBH,
Direct dyes	Direct Brown M, Direct Sky Blue 5B, Direct Black G, Sumilight Supra Blue B_2RL, Direct Red Brown RN
Kayacry dyes	Kayacry Ywllow 3RL, Kayacry Red GFL, Kayacry Blue RL, Kayacry Blue R,
Other dyes	Middle Black BL, Sili Yellow GN, Palatinech Blue GGN, Pakatubecg Tekkiw GRN.

Preparation of intact cells

The cells of bacteria were incubated in the medium containing dye for 24 hours in a rotary shaker (200 r/min) at 30°C and collected by centrifugation, washed twice with 1/15 mol. phosphate buffer at pH 7.0 and then suspended in the same buffer.

Measurement of products in the reaction of dyes with intact cells

Reaction products of both Diamond Chrome Red B(DCR-B) and Diamond Narry Blue RRN(DNB-RRN) with intact cells of Enterobacter D17 at 37°C were extracted with n-butanol. The solvent layer was detected with Beckman-7HS Spectrophotometer. Thin-layer Chromatography was carried out on a Wakogel F_{254} plate. The spots were analyzed by the ultraviolet (254 nm) method, and R_f of spots was calculated.

RESULTS AND DISCUSSION

Isolation and identification of decolorizing bacteria

400 strains of bacteria which are capable of decolorizing 5 kinds of dyes were isolated. 45 strains of them were able to decolorize activated dyes, Kayacry dyes, acidic mordant dyes and

direct dyes very well, then 13 strains of them were selected as excellent experimental strains for decolorizing dyes. The results are shown in Table 2.

Table 2 Dyes decolorized by bacteria

Strains of bacteria	Active dyes 10 kinds			Acid mordant dyes 13 kinds			Direct dyes 5 kinds			Kayacry dyes 4 kinds			Other dyes		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
D_{32}	7	0	3	10	1	2	4	0	1	3	0	1	2	2	0
D_{33}	7	0	3	10	1	2	4	0	1	3	0	1	2	2	0
D_4	7	0	3	10	1	2	4	0	1	3	0	1	2	2	0
D_8	1	0	9	4	5	4	0	3	2	4	0	0	0	0	4
D_{11}	1	0	9	3	2	8	0	2	3	4	0	0	0	2	2
D_{12}	0	0	10	2	1	10	0	0	5	3	0	1	0	0	4
D_{14}	2	0	8	8	3	2	1	1	3	4	0	0	0	1	3
D_{15}	0	0	10	1	0	12	0	0	5	2	0	2	0	0	4
D_{17}	3	0	7	8	2	3	0	3	1	2	0	2	0	1	3
D_{19}	0	0	10	1	0	12	1	0	4	1	0	3	0	0	4
D_{27}	2	0	8	3	1	8	1	1	3	4	0	0	0	1	3
D_{29}	1	1	8	8	3	2	3	0	2	2	0	2	0	1	3
D_{30}	0	0	10	1	0	12	0	2	3	3	0	1	0	0	4

1. good decolorization;
2. middle decolorization;
3. no decolorization.

Table 3 Decolorizing activities of intact cells of bacteria for Chrome dyes

Strains bacteria	Mordant Blue B dye removed*, ppm	Act**.	Diamond Chrome red B dye removed, ppm	Act.	Diamond Narry Blue RRN dye removed, ppm	Act.	Carmoisine B.2GL removed, ppm	Act.	Mordant Brown RH removed, ppm	Act.	Solo Chrome Grey B removed, ppm	Act.
Alteromonas												
D_{32}	37.5	0.84	111	2.47	95	2.12	100	2.23	8	0.27	93.7	2.1
Alteromonas												
D_{33}	53.7	1.3	115	2.55	117	2.0	112	2.46	21.5	0.48	102	2.27
Alteromonas												
D_4	40	0.89	113	2.5	104	2.31	100	2.22	22.5	0.5	88.7	1.97
Klebsiella												
D_8	/	/	69.8	1.53	73.8	1.64	80	1.78	17.5	0.39	61.3	1.36
Enterobacter												
D_{17}	26.3	0.58	50	1.11	52.5	1.12	96.3	2.14	25	0.56	72	1.6
Alceligenes												
D_{27}	0	0	105	2.33	91.3	2.03	78	1.73	18.8	0.42	31.3	0.7
Psuedomonas												
D_{30}	6.25	0.14	8.75	0.56	38.8	0.86	61.3	1.36	0	0	56.3	1.25

*: quantity of dye removed, ppm

**: decolorizing activity.

The 13 strains of decolorizing bacteria were identified as *Alteromonas* D_{32}, D_{33}, D_4; *Klebsiella* D_8, D_{14}; *Psuedomonas* D_{11}, D_{12}, D_{15}; *Enterobacter* D_{17}, D_{29}; *Corynobacterium* D_{19};

and *Alcaligenes* D_{27}. The strains of *Alteromonas* were isolated first from the aquatic sludge and their decolorization ability has not been reported before.

Activities of intact cells of bacteria for decolorization single dyes

Decolorizing reaction was carried out for 3 h at 37°C, pH 7, and 15 mg cell /ml for a dye concentration of 125 mg/L. The results are shown in Table 3 and 4. Activities of each of 7 strains in decolorizing the same dye were different. The decolorizing activity of *Alteromonas* for Mikacion Brilliant Red 58S is 2.5 mg dye /g cell.h. For decolorizing Mordant Blue B, activity of *Alcaligenes* D_{27} is only trace and that of *Alteromonas* D_{33} is over 1.2 mg dye/g cell.h. The color of Cibacron Brilliant Red K_2B could not be removed by *Psuedomonas* D_{30}, but it can be decolorized very well by the strains of *Alteromonas*. Under this experiment condition, Carmoisine B. 2 GL and DNB–RRN were decolorized easily by bacteria. *Alteromonas* D_{33}, D_{32}, D_4 have the highest decolorizing activities and can decolorize the most kinds of dyes in the experiments.

Table 4 Decolorizing activities of intact cells of bacteria for direct and active dyes

Strains of bacteria	Active Black KBH removed*, ppm	Act**.	Mikacion Brilliant Red 5BS removed, ppm	Act.	Cibacron Brilliant Red K_2P removed, ppm	Act.	Direct sky Blue 5B removed, ppm	Act.	Direct Red Brown RN removed, ppm	Act.
Alteromonas D_{32}	93.6	2.1	116	2.58	72.5	1.6	68.8	1.53	93.8	2.1
Alteromonas D_{33}	93.7	2.2	113	2.5	116.3	2.6	106	2.36	106	2.36
Alteromonas D_4	100	2.2	116	2.58	102.5	2.3	95	2.11	111	2.43
Klebsiella D_8	30	0.67	16.3	0.36	5.0	0.11	0	0	28.8	0.64
Alceligenes D_{27}	49	1.03	6.0	0.133	6.3	0.14	6.3	0.14	43.8	0.97
Enterobacter D_{17}	22.5	0.5	26.3	0.58	9.0	0.2	10	0.22	50	1.6
Psuedomonas D_{30}	31.3	0.69	0.25	0.05	0	0	8.75	0.19	41.3	0.92

*: quantity of dye removed, ppm

**: decolorizing activity.

Bacterial decolorization of mixed dyes

It is necessary to use some strains of bacteria with high activity in decolorizing mixed dyes for biological treatment of textile and dyeing wastewater containing mixed dyes. For this purpose, we examined characteristics of decolorizing mixed dyes (Table 5) by *Alteromonas* D_{32} with inoculation for 50h at 28°C, the results are shown in Fig.1, the rates of decolorizing 5 types of mixed dyes by strain D_{32} are more than 80% and only Kayacry mixed dyes appeared to have a longer time lag. These results indicated that it is suitable to use these strains for treating the textile wastewater containing various mixed dyes.

Conditions for decolorization of dyes

pH, temperature and oxygen are the important environmental factors for the growth of microorganisms. For this reason, studies of the effect of these factors of the microbial decolorization are very important for the biological treatment of textile wastewater.

Effects of pH on bacterial decolorization

The effects of different pH on the bacterial decolorization were carried out in the phosphate

buffer (1/15 mol/L) with different pH values and 15 mg cell/ml at 37°C for the dyes concentration of 50 ppm. The results (Fig.2) show that the optimum pH was 7–8 for decolorizing dyes by intact cells of *Alteromonas* D_{32}, D_4.

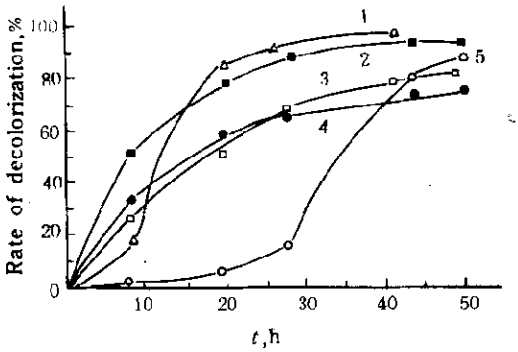

Fig. 1 Decolorization of mixed dye liquids by Alteromonas D 1. active mixed dyes; 2. direct mixed dyes; 3. direct, Kayacry, acidic mordant and active mixed dyes; 4. Kayacry mixed dyes; 5. active mixed dyes.

Table 5 The component of mixed dye liquids

Types of dyes	Names of dyes
Mordant mixed dyes	Mordant Blue B, Diamond Chrome Red B, Mordant Orange G,
Kayacry mixed dyes	Kayacry Red GL, Kayacry Blue R, Kayacry Yellow 3GL
Direct, Kayacry, Acid mordant, active mixed dyes	Mordant Blue B, Direct Key Blue 5B, Cibacron Brilliant Red K_2B, Kayacry Red GL
Direct mixed dyes	Benzo Bordearx GB, Direct Key Blue 5B Sumilight Supra Blue B_2RL
Active mixed dyes	Mikacion Brilliant Red 5BS, Cibacron Violet K-R Keamira Brilliant Orange KN-4R

Effect of temperature on bacterial decolorization

The test was carried out for 2h in the phosphate buffer with pH 7 at different temperature and 15 mg cell /ml. The results in Fig.3 indicated that the effect of temperature on the bacterial decolorization of dyes is important. The optimum temperature is 37°C for decolorization by intact cells of bacteria.

Effect of oxygen on decolorization of dyes

Alteromonas was cultivated under both static and shaking conditions in the nutrient medium containing different dyes. Removal time and the results are shown in Table 6. It was found that decolorizing rates of dyes were 4–9 times higher with static culture than with shaking culture, but the growth amount of cells were near 2 times more in the latter than in the former. It is clear that oxygen can promote the growth of bacteria but supressed the decolorization of dyes. These results are similar to those of Ogawa and Isaka (Ogawa, 1978).

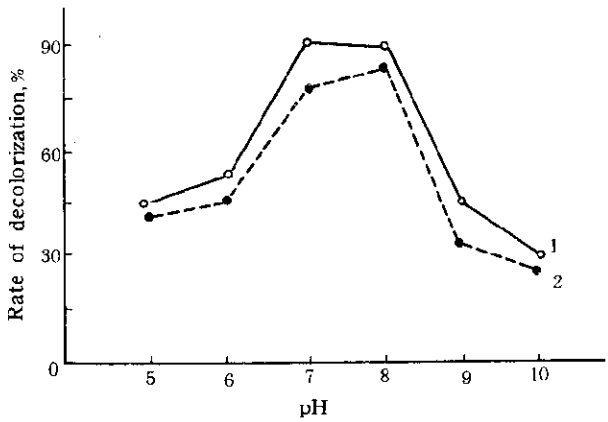

Fig. 2 Effect of pH on the decolorization of dyes by intact cells of bacteria, Mikacion Brilliant Red 5BS, 50ppm 1. Alteromonas D_{32}; 2.Alteromonas D_4

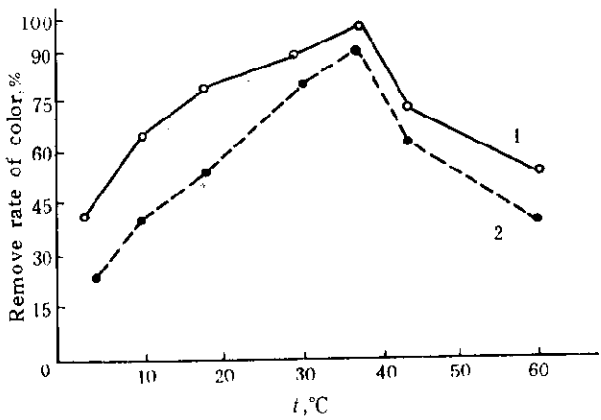

Fig. 3 Effect of temperature on the decolorization of dyes by intact cells of bacteria Mikasion Brilliant Red 5BS, 50ppm 1. Alteromonas D_{32}; 2.Alteromonas D_4

According to the previous results, the optimum conditions for biotreatment of textile wastewater containing dyestuffs ought to be controlled at 20–37°C and pH 6–9. Under the aerobic condition bacteria grow fast, but decolorizing rates of dyes decrease. Therefore, it is suitable to use facultative—aerobic process for biotreatment of textile and dyeing wastewater of degrade rapidly the dyes in the wastewater (Yang, 1987). In order to improve the growth of bacteria cells and to increase removal speed of dyes in wastewater, activated sludge from an aerobic reactor is returned into facultative reactor.

Table 6 The effect of oxygen on decolorization of dyes by Alteromonas D_{32}

Names of dyes	Culture conditions	Concentration of dyes, ppm, at 0 h	at 23 h	Rate of decolorization, %	Cell weight, g/L
Mikacion Brilliant Red 5BS	shaking	300	270	10	8.5
	static	300	18	94	3.0
Mordant Yellow GG	shaking	200	166	17	9.0
	static	200	58	71	3.0
Mordant Brown RH	shaking	300	300	0	6.0
	static	300	174	42	2.0

Elementary research in the mechanism of bacterial decolorization of dyes

In order to elucidate the mechanism of bacterial decolorization of dyes, UV absorption spectra and thin-layer chromatogram of n-butanol-extracted from mixture reaction products of decolorization of DCR-B and DNB-RRN by cells of Enterobacter D_{17} were measured. The results in Fig.4 and Fig.5 show that the intensity of maximum absorbances of DCR-B and DNB-RRN at 532 nm and 515 nm, respectively, decreases with time, whereas the intensity absorbance at near ultraviolet increases.

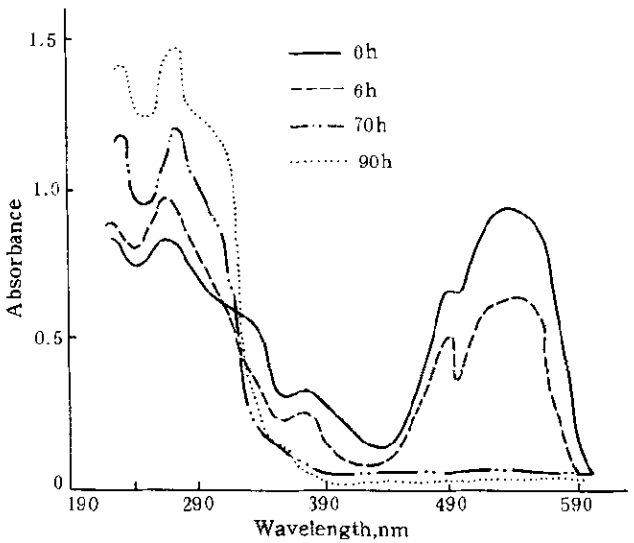

Fig. 4 UV spectra of the reaction products of Chrome Red B in n-butanol

This indicates that degradation production formed and the molecular structures of dyes changed.

The results from thin-layer chromatograms of the reaction products of dyes on silica are shown in Fig.6. Before decolorization, one spot appears and its R_f value is 0.55; after decolorization for 96h the spot of DCR-B disappears and two spots appear, their R_f are 0.87 and 0.89, respectively. Two spots of DNB-RRN appear and their R_f are 0.80 and 0.54, and other two spots appear and R_f values are 0.91 and 0.66, respectively, after decolorization. These results indicated that some dyes could be degraded and converted by bacteria.

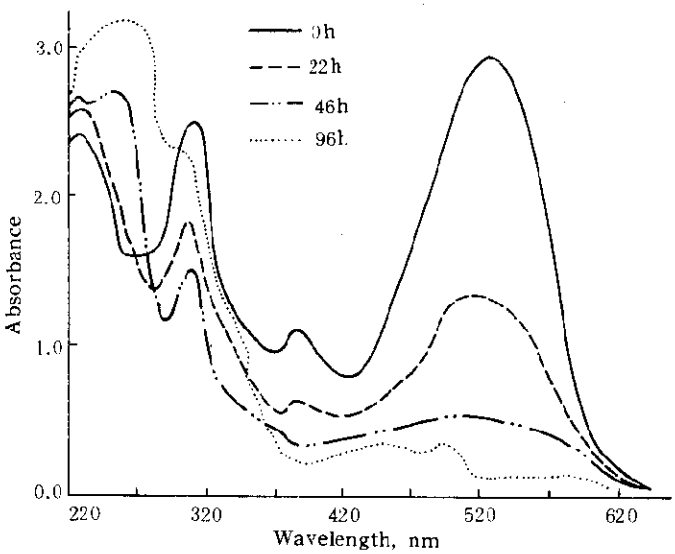

Fig. 5 UV spectra of the reaction products of diamond Narry Blue RRN in n-butanol

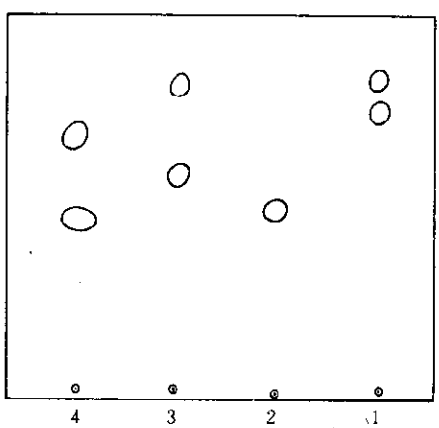

Fig. 6 Thin-layer chromatogram of the reaction products of dyes on silica gel , 1. Reaction products of diamond Chrome Red B; 2. Diamond Chrome Red B; 3. Reaction products of diamond Narry Blue RRN; 4. Diamond Narry Blue RRN, solvents; n-butanol: acetic acid: H_2O= 25:13:50(V/V).

CONCLUSIONS

The decolorizing strains of bacteria are excellent for the biotreatment of textile and dyeing wastewater because they possess high activities of decolorizing dyes.

The removal rate of color of dyes is 4–9 times better with static culture than with shaking. This result served as a microbial theoretical basis for efficient removal of color in the facultative treatment of textile and dyes wastewater.

It is proved that the dye can be decolorized by microorganisms through transformation and degradation of molecules of dyes. The metabolism pathway of dyes will be searched in the

future.

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