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Degradation of Synthetic Aromatic Textile Dyes by Native Bacteria Isolated from Textile Mill sites

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ABSTRACT

Hundred fifty bacterial isolates were obtained by enrichment culture from 12 samples collected from soil contaminated with dye effluents and effluent treatment plants of textile and dyeing industry at New Borg El-Arab, Cairo, Kafr El-Dawar, and El-Mehalla El-Kubra regions. The isolation of the microbial consortium was conducted on mineral salts medium (MSM) supplemented with three dyes. The microbial consortium was able to decolorize dyes, as evident by clearing the three azo dyes solutions under anoxic and anaerobic conditions. The isolates were checked for their ability to decolorize direct violet and reactive red dyes. The efficiency of the bacterial isolates in dye removal was investigated under aerobic and anaerobic conditions. The study revealed that no decolorization has taken place by isolates under aerobic conditions. The results revealed that the percentage of decolorization by the isolates reached 98.52 and 97.95 % of the original color of direct violet and reactive red dyes after 9 and 15 days, respectively, under anaerobic conditions. Twenty five isolates were selected as representative isolates based on the morphological and biochemical analyses. Two isolates are long rod, 6 isolates are short rods, and seventeen isolates belong to family enterobacteraece. The characterization of bacteria showed that the isolates belong to *Bacillus* and *Pseudomonas* sp. The degradation of the dyes is usually judged by the formation of aromatic amines. Most of the isolates under anoxic conditions were found to produce aromatic amines. This suggests the degradation of the dyes by the isolated bacteria.
Keywords: degradation, textile dyes removal, bioremediation, bacteria

INTRODUCTION

The world production of dyes is around 1 million tons, of which more than 50% are azo dyes (Stolz, 2001). These dyes are widely used in a number of industries, such as textile dyeing, food, cosmetics, and paper printing, among which the textile industry represents the largest consumer. All dyes do not bind to the fabric. Depending on the class of the dye, the dyes loss in wastewaters could vary from 2% for basic dyes to 50% for reactive dyes, leading to severe contamination of surface and ground waters in the vicinity of dyeing industries (O'Neill *et al.*, 1999). Azo dyes belong to the most important group of synthetic colorants. They are generally considered as xenobiotic compounds which are very recalcitrant to biodegradation (Padmavathy *et al.*, 2003a). The toxicity of azo dyes could lead to mortality, genotoxicity, mutagenicity, and carcinogenicity (Tan, 2001; Van der Zee, 2002). Microorganisms are known to play an important role in the mineralization of biopolymers and xenobiotic compounds like azo dyes (Lie *et al.*, 1998, Abd El-Rahim *et al.*, 2003). The mineralization of azo dyes are difficult under aerobic conditions because these dyes are recalcitrant and the electron-withdrawing character of the azo group are less susceptible to oxidative processes (Jin li, 2001). But these compounds however, are mineralized by two–step process; first step is the reduction of azo bond under anaerobic, or anoxic conditions, by anaerobic, facultative, or microaerophilic bacteria, and the second step is the mineralization of the intermediates under aerobic conditions by facultative or aerobic bacteria (Jin li, 2001; Van der Zee, 2002; Isik and Sponza, 2004; Pandey *et al.*, 2007). Anoxic decolorization of various azo dyes was stated by mixed aerobic and facultative anaerobic microbial consortia (Kapdan *et al.*, 2000; Padmavathy *et al.*, 2003b; Khehra *et al.*, 2005; Moosvi *et al.*, 2005). The present study aims at the isolation and characterization of bacterial strains capable to degrade direct and reactive dye in liquid culture. This can help in developing bioremediation approach for the treatment of water contaminated with textile dyes.

MATERIALS AND METHODS

Enrichment and isolation of dye-decolorizing micro-organisms

Twelve wastewater samples number 1, 5, 6, 10, 11, 17, 19, 24, 28, 37, 41 and 42 collected from the waste disposal sites of textile dyeing industries were enriched in mineral salts medium (MSM) amended with textile dyes. The content of the medium (g/ l) is as follows: 3.6; Na_2HPO_4, 1.0; $(NH_4)_2SO_4$, 1.0; KH_2PO_4, 1.0; $MgSO_4.7H_2O$, 0.01; Fe (NH_4) citrate, 0.1; $CaCl_2.2H_2O$ and 10.0 ml of trace elements solution. The trace elements solution had the composition (mg/ l): 10; $ZnS0_4.7H_2O$, 3.0; $MnCl_2.4H_2O$, 1.0; $CoCl_2.6H_20$, 2.0; $NiCl_2.6H_2O$, 3.0; $Na_2MoO_4.2H_2O$, 30.0; H_3BO_3 and 1.0; $CuCl_2.2H_2O$. The final pH of the medium was adjusted to 7.0. Stock solutions of 50% (w/v) glucose and 25% (w/v) yeast extract were sterilized separately and were added to MSM to the final concentrations of 5.6 mM glucose and 0.25% (v/v) yeast extract (Sharma *et al.*, 2004). MSM was used in all studies unless stated otherwise. The sterilized liquid and solid MSM containing 200 mg l^{-1} of reactive red, direct brown, and

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acid red dyes were inoculated with wastewater samples (1.5% v/v) and incubated at 28± 2°C for two week. All treatments were done with and without thioglycolate (1g/ liter) to test the bioremoval under anoxic and anaerobic conditions.

Isolation and screening of dye-decolorizing micro-organisms

As mentioned previously the MSM liquid medium was inoculated with (1.5% v/v) of wastewater samples collected from waste disposal sites of the textile processing and dye-manufacturing units in and around the area (Table 1). The tubes were incubated at 28± 2°C under static conditions. After two weeks incubation, 0.1ml of the enrichment culture was plated on MSM-agar containing 200 mg/l reactive red. The morphologically distinct bacterial isolates showing clear zones due to decolourization of the dye were selected for further studies. The bacterial isolates were subjected to identification testes. The pure cultures of these isolates were stored at 4°C on (MSM modified-agar without dye) slopes. These isolates were screened for their ability to decolorize the direct violet and reactive red dyes in liquid culture. A loopful of growth from stock culture slope was inoculated into MSM modified medium: [2.6g/l glucose, 1g/l $(NH_4)_2SO_4$, 0.1g/l NaCl, 0.5g/l $MgSO_4.7H_2O$, 1g/l K_2HPO_4, 0.5g/l KH_2PO_4, and 0.2g/l yeast extract] liquid medium and the flasks were incubated at 28 ± 2°C under static conditions. After 72 hours of incubation, two ml of the culture broth was transferred to tubes of 20 ml volume containing 300 mg/l of direct violet or reactive red, 0.1g/l NaCl, 0.5g/l $MgSO_4.7H_2O$, 1g/l K_2HPO_4, 0.5g/l KH_2PO_4, and 0.2g/l yeast extract. The tubes were filled completely and sealed with screw caps so as to achieve anoxic conditions as described by Manjinder *et al.* (2005). The number of tubes incubated corresponded to the number of samples needed throughout the whole experiment so that each tube is opened only once. The dyes removal was judged by decolorization efficiency of the solution. Therefore, the optical density (O.D.) of the culture filtrates was measured at intervals of 2, 3, 4, 5, 7, 10, 12 and 15 days at wavelength 555 nm for reactive red dye and at intervals of 3, 6, and 9 days at wavelength 542 nm for direct violet dyes. The optical densities of samples containing dye were used to calculate the percentage of color removal as described in equal. The uninoculated control was also incubated to check the abiotic decolorization of dye. The decolorization efficiency of the isolates was determined as described previously.

Identification and characterization of the bacterial isolates:

The identification of the reactive red and direct violet dye-degrading bacteria was conducted according to Bergey's Manual of Determinative Bacteriology (1994). The morphology and motility of the bacterial strains were determined by conventional methods. Physiological and biochemical characterizations were performed using the API 20 E Kit

Determination of aromatic amine:

Three methods were used for detecting aromatic amines. The first method is based on detection and identification of aromatic amines by direct UV spectrophotometer using standard aromatic amine (sulfanilic acid) by Jenway UV. Visible- 2605 spectrophotometer, England (Isik and Sponza, 2003a; Pinheiro *et al.*, 2004; Bhaskar *et al.*, 2003).

In the second method, two steps were performed. The first gives solution (A): HCL was added to 0.5 ml of

$$\text{Decolourization\%} = \frac{\text{Reading of control decolourization} - \text{Reading of sample decolourization}}{\text{Reading of control decolourization}} \times 100$$

Table (1): Sources and some properties of wastewater samples

Factory site	Company name	Sample No.	Place of sampling	BOD*	TSS*	pH	EC*
New Borg El-Arab	Seif El-Din Co. For Industry & Trade	**1**	Outside the drainage lugoone	150	6020	11.6	2595
	Egyptian Int'l Co. For Knitting & Dyeing (Dyetex)	**5**	The dyehouse	250	6376	9.27	5196
		6	Outside the drainage lugoone	50	748	5.82	192
	Egyptian Industries Co. (Sougic)	**10**	Outside the drainage lugoone	360	800	8.80	557
		11		180	1120	7.22	845
Cairo	El-Mukatem Dyehouse	**17**	The drainage water of the dyehouse	400	2728	10.7	1472
	Shams Company	**19**	Outside the drainage.	90	352	8.77	224
Mehalla Kubra	El-Salam Textile & Dyeing Co.	**24**	The drainage canal	120	1900	7.26	1875
Shubra El Khima	El-Salam Dyeing & Finishing Co.	**28**	Outside the drainage canal.	600	964	3.69	768
	Small Dafshu El-Beida drain	**37**	The small Dafshu El-Beida drain	150	1200	3.8	998.4
Kafr El-Dawar	Misr El-Beida Dyers	**41**	In and around the big Dafshu drain	Nd^+	724	7.50	666
		42	Outside the drainage canal	Nd^+	528	6.76	781

* Values ppm, TSS= Total soluble solids, Nd= not detected, BOD= Biological oxygen demand.

treated sample to adjust pH at 1-2. Few drops of Sod. Nitrite solution (2% $NaNO_2$) were added to the sample at 0- 4ºC, and stirred for production of diazonium salt. After incubation for 15min with stirring at 0-4ºC, nitrous was tested by iodide starch paper (+ve is blue colour, -ve is no colour), and excess nitrous was adjusted by adding very few gms of sulphamic acid. For the preparation of coupler (solution B), such as β-naphthol, or phenol. 1.0g (β-naphthol) was dissolved in 100ml H_2O and pH was adjusted at 9-10 by NaOH solution at 0- 4ºC. Few drops of (A) were added to few ml of (B) in a test tube and pH was adjusted at 8-9. A positive (+ve) reaction giving colour means that aromatic amines are found, -ve colour (absence of aromatic amine).

In the third method, 30 mg of anhydrous aluminum chloride ($AlCl_3$) were put in a 3 inch test tube and gently heated over a microflame until $AlCl_3$ sublimed on the walls of the test tube. The tubes were then cooled in air for 30 sec and then 1-2 drops of a solution of the unknown sample were flowed down the wall of the test tube while rotating the tube. A color will develop on the wall of the test tube indicating the presence of aromatic amines. Simple benzene derivatives give a yellow-orange or red color; bicyclic aromatics give a blue or purple color; and more complex aromatic systems give a green color. The reaction involves -complex formation between the aromatic system and $AlCl_3$ (McMurry, 2000).

RESULTS

Populations of microorganisms in the Wastewater samples grown on Remazol blue dye

Suspension from eight effluents collected from textile factories dumping sites were plated separately on mineral salt agar medium (MSM) containing 0.2 g/l of remazol blue dye and incubated for 48h. The dye was supplemented to the medium before autoclaving. The results in Table (2) show that the counts of bacteria, fungi, and actinomycetes on petri dishes ranged between 3 to 262 colonies /ml wastewater. The results indicate that bacteria, fungi, and actinomycetes counts were the highest in sample number 11, being 106, 86, and 70 colonies, respectively. However, bacteria dominated the colonies in the wastewater sample number 41 being 230 CFU/plate. The fungal counts were 5 and 58 in samples 17 and 37, whereas the actinomycetes gave 5 and 4 colonies with the wastewater samples number 17 and 28, respectively.

Table (2): Microbial counts (CFU)/ petri dishes of 8 wastewater samples grown in Remazol blue dye.

Samples No.	Bacteria	Fungi	actinomycete	Total counts
5	24	-	-	24
11	106	86	70	262
17	7	5	5	17
24	3	-	-	3
28	22	-	4	26
37	73	58	-	131
41	230	-	-	230
42	46	-	-	46

Enrichment and isolation of dye-decolorizing microorganisms

Selective enrichment of 12 wastewater samples led to the isolation of a microbial consortium from MSM/broth medium supplemented with 200 ppm reactive, direct, and acid dyes. The microbial consortium was able to decolorize dye, as evident by clearing the three azo dyes solutions under anaerobic and anoxic conditions (Fig. 1). Bioremoving of reactive dye under anoxic and anaerobic conditions is listed in Table (3). The removal percentages under anoxic and anaerobic conditions ranged between 39.2-96.4% and 37.2-94.6%, respectively, with six wastewater samples (Table 3). The results are different with using other wastewater samples number 1, 11, and 28 where no removal of reactive dye was observed under anaerobic and anoxic conditions (Table 3). No removal of direct brown and acid dyes was recorded under anaerobic and anoxic conditions with samples number 1, 10, 11, and 19. And no removal was recorded under anaerobic conditions with samples number 28, and 42. The

Table (3): Percentages of three textile dyes Removal by the consortium of bacteria under anoxic and anaerobic conditions

No. of waste-water sample	Reactive red		Direct brown		Acid red	
	Anoxic conditions	anaerobic conditions	anoxic conditions	anaerobic conditions	anoxic conditions	anaerobic conditions
1	0	0	0	0	0	0
5	39.2	94.6	67.0	69.9	94.6	86.7
6	84.0	88.0	62.4	84.0	92.6	80.5
10	92.1	90.8	0	0	0	0
11	0	0	0	0	0	0
17	0	37.2	38.9	59.2	42.7	0
19	0	87.2	0	0	0	0
24	96.4	94.6	65.2	72.9	92.9	91.8
28	0	0	30.9	0	92.0	0
37	92.8	86.9	79.5	56.8	94.9	0
41	90.3	86.6	71.7	70.0	93.9	0
42	86.2	0	74.2	0	86.9	0

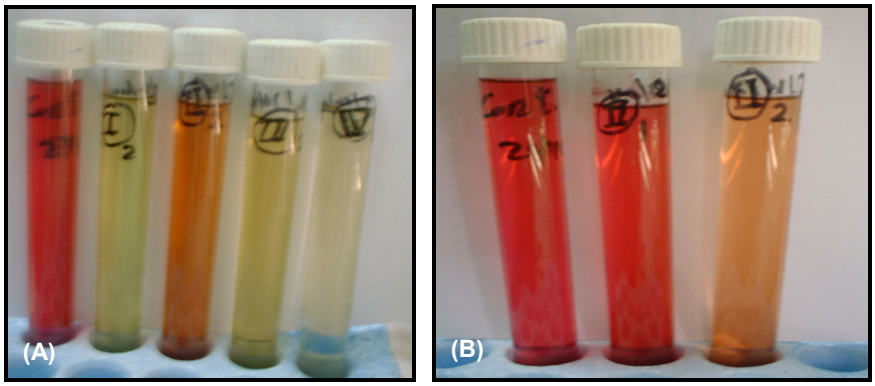

Figure (1): (A and B) Removal of Remazol red dye using enrichment cultures of four wastewater samples under aerobic and anaerobic conditions.

microbial consortium was also investigated for its ability to decolorize direct brown dye. The overall decolorization of 30.9 and 56.8 to 79.5, and 84.0% was achieved up to an initial dye concentration of 200 mg/l in one week under anoxic and anaerobic conditions respectively (Table 3) whereas, with acid dye, the decolorization efficiency of the consortium increased as 42.7 and 80.5 to 94.9 and 91.8%, respectively. Removal activity was minimal under anoxic and anaerobic conditions with sample 17 when used direct brown and acid dyes. The morphological identification of bacterial consortium and the results of other dye removal under anoxic conditions are described in the next experiments.

Isolation and screening of dye-decolorizing bacteria

A total of 150 bacterial isolates were isolated from samples collected from Seif El Din Co. For Industry & Trade, Egyptian Industries Co. (Sougic), Egyptian Int'l Co. For Knitting & Dyeing (Dyetex**) (**New Borg El-Arab region**)**, El-Mukatam Dyehouse, Shams Company **(**Cairo**)**, Misr El Beida Dyers **(**Kafr El-Dawar**),** and El-Salam Textile & Dyeing Co. (Mehalla Kubra**).** The isolates were screened for their ability to decolorize reactive red and direct violet dyes at concentration of 300 mg/l in MSM developed broth. The results are presented in Tables (4) and (5) and (Figures 2 a, b, c and d). The efficiency of the bacterial isolates in dye removal was investigated under anoxic conditions as no ability to decolorize the dyes was achieved under aerobic conditions.

Most isolates from El-Mokatam Dyehouse wastewater samples failed in decolorizing the added reactive red dye until 10 days incubation. However, the removal percentage reached 86.40 and 93.28% with isolate B1, and B15 after 15 days incubation, respectively. Whereas the Shams Company isolates gave removal percentages reaching 97.0% from reactive red dye for isolates number S15 and S19 (Table 4).

The isolates from New Borg El-Arab area specifically, Dyetex Company, didn't show decolorization capacity till four days of incubation except isolate A22, which recorded highly efficient decolorization of reactive red dye being 95.69 and 96.69% after 4 and 15 days of incubation, respectively. The isolate number H1 obtained from Egyptian Industries Company (Sougic) wastewater samples were found to decolorize the added reactive red dye after 15 days of incubation (Table 4). The isolates collected from Seif El Din Co. for Industry & Trade were failed to remove reactive red dye, except isolates number F24, which had 95.36% removal, as shown in Table (4). The isolates were isolated from soil sample collected from Sougic Company at New Borg El-Arab Region. The decolorization of reactive red was not observed in the early days of incubation except isolate R2 (Table 4) which is considered the best isolate in the decolorization of dye after one day of incubation. A minimum of 26.20 and 48.02% reactive red color removal were obtained with two isolates number Y4 and M4 from Kafr El-Dawar and El-Mehalla El-Kubra regions after 15 days incubation (Table 4).

Table (5) shows the percentage of decolorization of direct violet dye by bacterial isolates (50 isolates) collected from two sites at Cairo region, namely El-Mokatam Dyehouse and Shams Company. The decolorization reached 88.04 and 98.52 % of the original color of direct violet (300 mg/l) after 9 days of incubation. The most efficient isolates were S19 and B15 isolated from Shams Company and Al Mokatam Dyehouse, respectively (Table 5). Most of the isolates from both sites could remove about 60 % of direct violet dyes after 9 days of incubation.

In case of direct violet dye, the bacterial isolates from New Borg El-Arab region (88 isolates) were capable to decolorize 3.45 to 94.78 % of dye. The best 4 isolates collected from (Dyetex) and (Sougic) Company in decolorization of direct violet dye are isolates: A22, A17, A26, and H1 which decolorized the dye in amounts of 87.67, 89.78, 90.25%, and 94.78%, respectively after 9 days of incubation (Table 5).

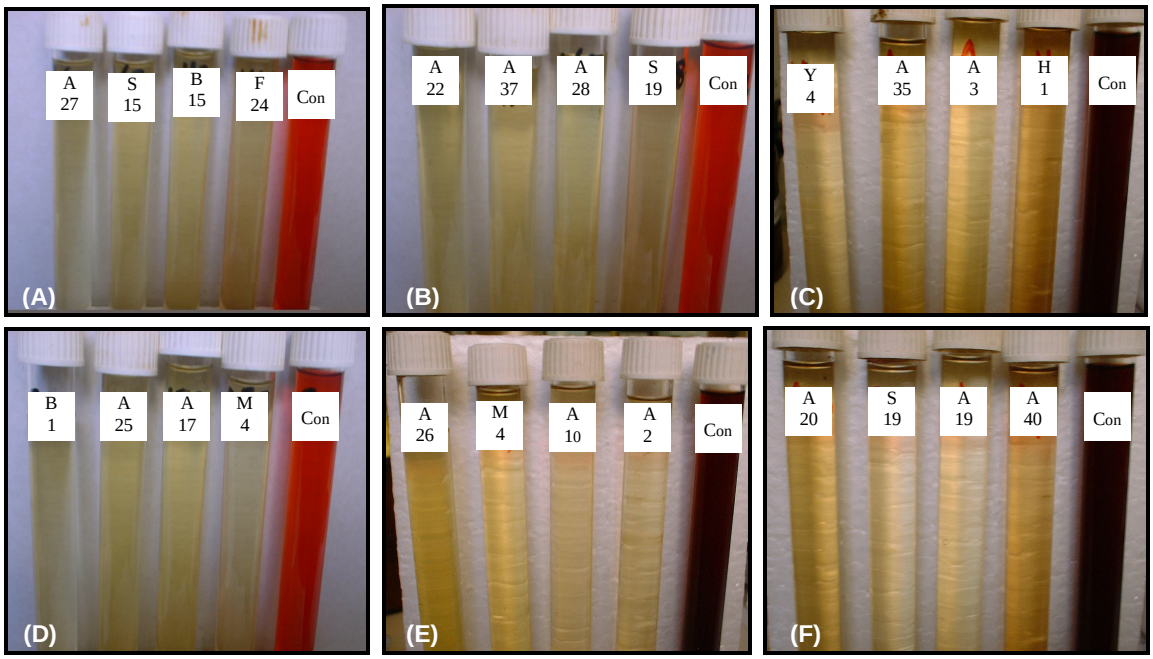

Figure (2): (**A**, **B**, **C**, **D**, **E**, **F**) Removal of reactive and direct dyes by bacterial isolates obtained from New Borg El-Arab, Cairo, Mehalla Kubra and Kafr El-Dawar) under anoxic conditions.

Table (4): Decolourization percentage of reactive red dye by bacterial strains isolated from different sites

Serial No.	Isolates No.	Decolourization %							
		2 days	3 days	4 days	5 days	7 days	10 days	12 days	15 days
		Dyetex company (New Borg El-Arab)							
1	A2	0.0	0.0	11.50	34.27	55.11	55.11	72.34	89.90
2	A3	0.0	0.0	14.74	26.82	47.16	47.16	69.98	82.62
3	A17	0.0	0.0	09.93	50.09	76.80	80.00	90.50	96.09
4	A19	0.0	0.0	0.0	10.13	31.10	67.51	83.25	96.97
5	A20	0.0	0.0	09.76	30.54	79.90	89.98	96.45	96.45
6	A21	0.0	0.0	13.80	31.38	83.31	90.67	97.95	97.95
7	A22	31.44	75.88	95.69	95.69	96.69	96.69	96.69	96.69
8	A24	0.0	0.0	17.46	76.32	96.46	96.46	97.38	97.38
9	A25	0.0	08.43	39.69	80.44	94.85	95.58	96.93	96.93
10	A26	0.0	0.0	0.0	34.07	46.69	70.43	84.88	94.07
11	A27	0.0	0.0	13.79	30.86	48.46	68.90	86.03	97.13
12	A29	0.0	0.0	16.79	75.00	97.38	97.95	97.95	97.95
13	A35	0.0	0.0	27.69	65.89	80.56	85.50	91.27	96.72
14	A37	0.0	0.0	14.09	68.56	80.41	86.14	90.65	97.10
15	A40	0.0	0.0	0.0	08.80	28.59	59.38	82.80	87.08
16	A41	0.0	0.0	50.40	80.24	93.64	93.64	93.64	93.64
		Safe El-Dean Company (New Borg El-Arab)							
17	F24	0.0	0.0	12.13	48.12	77.84	84.33	90.34	95.36
		Al-Mokatam dyehouse (Cairo)							
18	B1	0.0	0.0	09.70	40.60	71.58	75.90	80.45	86.40
19	B15	0.0	0.0	0.0	17.65	30.64	70.00	82.56	93.28
		Wastewater from Sougic Company (New Borg El-Arab)							
20	H1	0.0	0.0	05..31	18.65	30.23	31.49	40.61	73.78
		Soil sample (Sougic company) (New Borg El-Arab)							
21	R2	26.42	28.17	40.68	51.11	74.86	78.90	80.70	84.67
		El-Shams Company (Cairo)							
22	S15	0.0	0.0	0.0	12.95	30.51	44.59	76.91	97.84
23	S19	0.0	10.87	34.74	59.98	74.73	94.56	97.13	97.13
		Misr El-Beida Dyers (Kafr El-Dawar)							
25	Y4	0.0	0.0	0.0	0.0	0.0	07.65	15.40	26.20
		El-Salam plant (El-Mahalla El-Kubra)							
25	M4	0.0	0.0	0.0	0.0	0.0	05.64	25.81	48.02

Similar results were also obtained from two superior isolates from Misr El-Beida Dyers at Kafr El-Dawar region and from El-Salam Textile & Dyeing Co. at El-Mahalla El-Kubra numbers Y4 and M4, which could remove the direct violet dye after 9 days of incubation with the removal percentages above 90% of the added dye (Table 5). The isolates collected from Seif El Din Co. For Industry & Trade did not show any efficient removal of direct dye since the removal percentage did not exceed 21.00%. This study clearly shows that very high decolorization efficiencies were achieved under anaerobic incubations. The most efficient 25 bacterial isolates in bioremoval of direct violet and reactive red dyes (Table 6) were subjected to morphological and biochemical characterization.

The results of routine morphological and some physiological characteristics of the isolated bacteria are summarized in Table (6). The 25 bacterial isolates filled within Gram positive, Gram negative motile, and anaerobic bacteria. Based on the morphology of the isolates, 23 of them were short rods and Gram negative respectively, and 2 isolates were long rods and gram positive. The isolation of efficient dye decolorization from samples collected from the waste disposal sites indicates the natural adaptation of these microorganisms to survive in the presence of the toxic dyes.

Table (5): Decolourization percentage of direct violet dye by bacterial strains isolated from different sites of stagnant water lagoon

Serial No.	No. of isolates	Decolourization % 3 days	6 days	9 days
		Dyetex company (New Borg El-Arab)		
1	A2	68.61	78.99	83.63
2	A3	67.96	75.73	86.33
3	A17	67.27	82.67	89.78
4	A19	76.15	85.11	86.44
5	A20	61.05	83.78	86.67
6	A21	78.95	82.80	85.10
7	A22	80.45	84.53	87.67
8	A24	79.11	83.42	85.52
9	A25	82.59	83.89	85.69
10	A26	79.05	84.95	90.25
11	A27	81.14	82.80	85.39
12	A29	76.96	78.53	81.14
13	A35	75.99	80.75	84.22
14	A37	62.58	75.18	84.37
15	A40	76.51	80.37	84.56
16	A41	76.78	78.92	83.81
		Safe El-Dean Company (New Borg El-Arab)		
17	F24	06.47	13.38	21.00
		Al-Mokatam dyehouse (Cairo)		
18	B1	44.90	80.35	80.96
19	B15	66.43	86.18	88.04
		Wastewater from Sougic Company (New Borg El-Arab)		
20	H1	84.10	94.78	94.78
		Soil sample (Sougic company) (New Borg El-Arab)		
21	R2	48.23	59.98	67.22
		El-Shams Company (Cairo)		
22	S15	35.41	54.74	67.54
23	S19	78.95	88.27	98.52
		Misr El Beida Dyers (Kafr El-Dawar)		
24	Y4	52.97	73.61	90.24
		El-Salam plant (El-Mahalla El-Kubra)		
25	M4	64.79	76.82	91.62

Biochemical characteristics

Bacterial isolates showed catalase, oxidase activity (Group3, 4), as well as nitrate reductase No hydrolysis of gelatin were found. The bacterial isolates acidification of glucose is shown in Table (6). The results obtained with API 20 E Kit (Table 6) indicated that group 1 included 10 bacterial isolates similar to *Enterobacter cloacae*, with % id = 94.3 to 97.5, group 2 contained 8 bacterial isolates similar to *Enterobacter sakazakii*, with % id = 91.1 to 92.1. Group three contained 5 bacterial isolates similar to *Pseudomonas* spp, with % id = 77, and the last group included 2 bacterial isolates similar to *Bacillus* spp, with % id = 97.1.

Determination of aromatic amine

The degradation of the dye by bacteria is judged by the formation of aromatic amines as key compounds in the degradation process. In this study, three procedures were used to determine the aromatic amine, produced by degradation of reactive red dye by the 25 bacterial isolates. The first method reactive red dye gave a peak at maximum wavelength 555 nm, whereas standard aromatic amine gave a peak at maximum wavelength 294 nm. The results show that 19 samples after

Table (6): Morphological and biochemical characterization of 25 bacterial isolates capable of direct and reactive dyes degradation

No. of Isolates	Gram stain	Morphology	Spore forming	Oxidase activity	Catalase activity	Oxidation- fermentation test: Aerobic Growth	Aerobic pH change	Anaerobic Growth	Anaerobic pH change	Nature of microbe
A_2	+	Long rod	+	+	+	+	-	-	-	Aerobic
A_3	-	Short rod	-	+	+	+	Acid	+	-	facultative
$A_{17, 19, 20, 21, 22, 24, 25, 27, 29, 35, 40}$, B_{15}, M_4, Y_4	-	Short rod	-	-	+	+	Acid	+	Acid + gas	facultative
$A_{26, 37}$, S_{15}, S_{19}	-	Short rod	-	+	+	+	Acid	+	Acid + gas	Facultative
A_{41}, B_1	-	Short rod	-	-	+	+	Acid	+	Acid	facultative
H_1	-	Short rod	-	-	+	+	Acid	+	acid	facultative
R_2	+	Long rod	+	+	+	+	-	+	Acid + gas	facultative
F_{24}	-	Short rod	-	+	+	+	Acid	-	-	Aerobic

reduction gave a peak at maximum wavelength 275 nm. This indicates the biodegradation of reactive red dye to aromatic amines.

Using the second method, isolates produced aromatic amines as shown in Table (7) and Figure (3). Nineteen bacterial isolates gave the aromatic amine peaks at wavelength 275 nm (Fig. 4). The peak of dye was formed after coupling the aromatic amine with n-(1-naphthyl) - ethylenediamine recorded at 345 nm.

In the third method, the same nineteen bacterial isolates produced aromatic amine, as shown in Table (7) and Figure (3).

The removal of dye color in addition to the formation of aromatic amines are indicative to the degradation of the dye.

Discussion

Many studies emphasized the role of bacteria in the degradation of textile dyes. Using the culture enrichment approach, one hundred and fifty dye-decolorizing bacteria were isolated on MSM modified broth from textile mill sites. The isolates (each isolate separately) were screened for their ability to decolorize reactive red and direct violet dyes at a concentration of 300 mg/L in the broth medium. Manjinder *et al.*, (2005) using the same enrichment technique, isolated bacterial strains capable of decolorizing azo dyes present in soil/sludge samples collected from waste disposal sites of local textile industries. The isolation of efficient dye decolorization from samples collected from the waste disposal sites indicates the natural adaptation of these microorganisms to survive in the presence of these toxic dyes. Chen *et al.* (2003) also isolated microorganisms capable of decolorizing different azo dyes from sludge samples collected from lake-mud and wastewater treatment plant.

Table (7): Production of aromatic amines by the bacterial isolates using two procedures

Isolates No	Dizotization method	$ALCL_3$ method
A2	+	+*
A3	-	-
A17	+	+
A19	-	-**
A20	-	-
A21	-	-
A22	+	+
A24	+	+
A25	+	+
A26	-	-
A27	+	+
A29	+	+
A35	-	-
A37	+	+
A40	+	+
A41	+	+
B1	+	+
B15	+	++
F24	+	+
H1	+	+++
M4	+	+
R2	+	+
S15	+	+
S19	+	+
Y4	+	+

* +, density of red color formed after the dye
**-, no aromatic amine formed

The efficiency of the bacterial isolates in dye removal under anoxic conditions showed the ability to decolorize the dyes only under such anoxic/anaerobic conditions. Khehra *et al.* (2006) reported that the main color removal takes place under the anaerobic conditions. Several reports showed that the color removal efficiency reached above 95% under anoxic condition and the contribution of aerobic phase to color removal was negligible (Robinson *et al.,* 2001; Isik and Sponza, 2004).

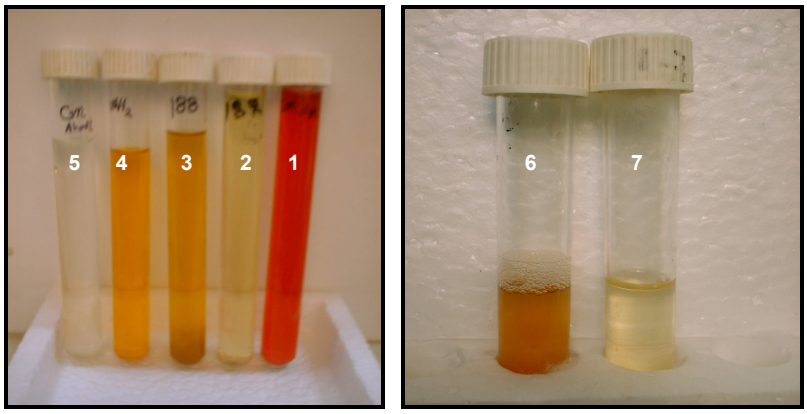

Figure (3): Production of aromatic amines as indication to Reactive red dye biodegradation. **1:** strain S19 with reactive red dye, **2:** degradation of reactive red dye bye bacterial strain (S19), **3:** azo dyes are produced by coupling a diazonium salt of aromatic amine, **4:** azo dyes are produced by coupling a n-(1-naphthyl)- ethylenediamine (aromatic amine, control) **5:** Positive Control as n-(1-naphthyl)- ethylenediamine (aromatic amine), **6:** Treated samples give the orange color at tested with AlCl3, and **7:** Control no color observed with MSM+Alcl3.

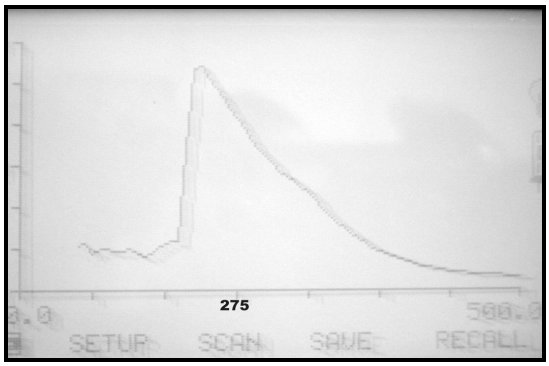

Figure (4): Formation of aromatic amines as a result of dye biodegradation (Aromatic amine peak at UV spectrophotometer at 275nm).

In this study, 25 isolates were selected from 150 isolates. These isolates under anoxic conditions recorded high decolorization percentages of Reactive Red dye ranging between 26.20% for isolate Y4 isolated from Misr El Beida Dyers at Kafr El-Dawar to 97.95% for isolate A29 isolated from Safe El-Dean Company at New Borg El-Arab. It is likely that bacteria decolorize and degrade azo dye by sequential anaerobic/anoxic conditions and aerobic conditions in the medium, the color removal was 98% with synthetic dye wastewater (Khehra *et al.*, 2005) and 60% color removal with real textile wastewater (Kapdan and Alparslan, 2005). Yang *et al.* (2004) found that maximum tolerable dyestuff concentration by *P. sordida* ATCC90872 was 200 mg/l. Total organic concentration (TOC) removal efficiency was around 80% for dyestuff concentrations between 50 and 200 mg/l and decreased to 52% at an initial dyestuff concentration of 400 mg/l. higher dyestuff concentrations could have toxic effects on fungi. Although in the present study many of the isolates were able to grow aerobically, the decolorization was achieved only under anaerobic conditions. Pure bacterial strains, such as *Pseudomonas luteola, Aeromonas hydrophila, Bacillus subtilis, Pseudomonas sp. and Proteus mirabilis,* were able to decolorize azo dyes under anoxic conditions (Chang *et al.*, 2001; Chen *et al.*, 2003; Yu *et al.*, 2001). Azo dye decolorization by mixed and pure cultures generally required complex organic sources, such as yeast extract, peptone, or a combination of complex organic source and carbohydrate (Chen *et al.*, 2003; Khehra *et al.*, 2005).

On the other hand, the isolate A29 from Safe El-Dean Company at New Borg El-Arab did not remove the direct violet dye as obtained with reactive red dye. The percentage of decolorization by this isolate did not exceed 21% of direct violet dye. Abd El-Rahim *et al.*, (2003) found that the percentage of decolorization by some isolates of bacteria and actinomycetes did not exceed 20 and 25%, respectively, of the original color of direct yellow and Erio red dyes after 21 days of incubation.

The formation of aromatic amines is the key step in the degradation of azo dyes. In this work, three procedures to determine the aromatic amines, produced by degradation of reactive red dye by the 25 bacterial isolates were used. The spectrophotometric analysis showed that the reactive red dye gives a peak at maximum wavelength 555 nm, whereas standard aromatic amine gives a peak at maximum wavelength 294 nm. (Kalyuzhnyi *et al.*, 2000; Isik and Sponza, 2003b; Perez, 2001). Pinheiro *et al.* (2004) reported that the aromatic amines could be measured by spectrophotometer at wave lengths ranging from 190-406 nm.

The results show that the degradation product of 19 strains gave a maximum peak at wavelength 275 nm. This indicates the biodegradation of reactive red dye to aromatic amines. These isolates must contain azoreductase enzyme and can reduce azo bond to produce aromatic amine. Pandey *et al.* (2007) reported that the reductive cleavage of azo bond, leads to the formation of aromatic amines. This represents the initial reaction during the bacterial degradation of azo dyes. Sandhya *et al.* (2005) reported that azo dyes are decolorized under anaerobic or microaerophilic conditions by the enzyme azoreductase secreted by microorganisms. Kalme *et al.* (2007) reported that the final product of azo dye biodegradation under static anoxic condition was aromatic amines such as 4-amino naphthaline and amino naphthaline sulfonic acid. The aromatic amine resulted from azo dye reduction is usually very toxic and might be carcinogenic (Mansour *et al.*, 2007).

In conclusion large number of isolates from textile dye effluent were found to be capable in degradation of direct and reactive dyes. These isolates were identified using API E20 and were found to belong to *Enterobacter cloacae* and *Enterobacter sakazakii species*. Production of aromatic amines as primary compounds in degradation of the synthetic aromatic dyes showed that 19 isolates out of 25 were able to produce the aromatic amine under anoxic condition. This indicates the capacity of these isolates to degrade the dyes.

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التكسير الحيوى للصبغات العطرية النسجية باستخدام بكتيريا مسطوتنة ومعزولة من مخلفات الصناعات النسجية

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الملخص العربـــى

يهدف البحث إلى دراسة المعالجة الحيوية والتكسير الحيوى لبقايا الصبغات النسجية. وذلك بالحصول على عزلات بكتيرية عن طريق مزارع الإكثار لإثنى عشر عينة جمعت من مناطق ملوثة بمخلفات الصبغات تشمل عينات تربة وعينات مخلفات مصانع النسيج والصباغة من منطقتى برج العرب والقاهرة. تم الحصول على خليط الميكروبات على بيئة (MSM) مضاف اليها ثلاث صبغات مختلفة والخليط الناتج له القدرة على ازالة لون الصبغات تحت الظروف اللاهوائية والأنوكسيك (Anoxic) وتم عزل 150 عزلة بكتيرية واختبار كفائتها على ازالة صبغتى ال Reactive red وDirect violet تحت الظروف الهوائية والاهوائية واوضحت الدراسة عدم حدوث ازالة اللون تحت الظروف الهوائية وتمت عملية ازالة اللون تحت الظروف اللاهوائية وصلت نسبة ازالة اللون الى 97.95 و 98.52 % فى صبغتى Reactive red وDirect violet بعد15 و 9 يوم على الترتيب .

تم إختيار 25 عزلة كأفضل العزلات كفاءة وصنفت بالطرق المورفولوجية والبيوكيماوية فكانت عزلتين long rod ، وثلاثة وعشرون short rod. وصنفت هذه العزلات على أنها عزلتين من جنس *Bacillus* sp. و 6 عزلات *Pseudomonas* sp. و17 عزلة *Enterobacter* sp. وأكدت الدراسة أن معظم العزلات البكتيرية تكسر الصبغات تحت الظروف الاهوائية وينتج عن هذا التكسير أمينات عطرية . و تقترح هذه الدراسة استخدام هذه السلالات البكتيرية المعزولة لتكسير الصبغات تحت الدراسة.