JOURNAL OF PURE AND APPLIED MICROBIOLOGY, December 2012. Vol. **6**(4), p. 1559-1570

Microbial Reduction of Monoazo and Diazo-linked Dyes by *Pseudomonas aeruginosa* and *Pseudomonas putida*

Eiman Rahnema Falavarjani*, Alireza Chackoshian Khorasani and Seyyed Mohamad Ghoreishi

Department of Chemical Engineering, Isfahan University of Technology, Isfahan, Iran.

(Received: 15 June 2012; accepted: 30 July 2012)

The microbial decolorization was investigated for chromophores removal as an effective and potential method to be applied in textile industries. In this study, four different azo dyes and their mixture, C.I. Acid Blue 113 (AB-113), C.I. Basic Red 46 (BR-46), C.I. Direct Blue 151 (DB-151), C.I. Direct Brown 2 (DB-2), and mixture of four dyes (Mix) were subjected to biodegradation using *Pseudomonas aeruginosa*, and *Pseudomonas putida* at pH 7.2 and 30 °C. *P. aeruginosa* completely decolorized AB-113 at all initial dye concentrations, BR-46 at concentrations of 0.1 and 0.2 g/L and DB-2 at concentrations of 0.1, 0.2 and 0.5 g/L. *P. putida* completely decolorized AB-113 at concentrations of 0.1 and 0.2 g/L, DB-2 at concentrations of 0.1 and 0.2 g/L. Mixture of four dyes was also completely decolorized at concentrations of 0.1 g/L by *P. putida*. Decolorization processes followed first and second order kinetics with respect to dye concentration. The higher calculated rate constants of first and second order were observed at low dye concentrations.

Key words: Monoazo dyes, Diazo dyes, *Pseudomonas aeruginosa, Pseudomonas putida*, Decolorization, Kinetic model.

One of the important challenging probleme is degradation of dyes from textile wastewater[1]. About 10000 different dyes are being used in industries and worldwide consumption of dyes is over 7×tonnes/year[2,3].

Dyes include several various types such as acidic, reactive, basic, disperse, azo, diazo, anthraquinone-based and metal–complex, sulfur, indigoid, triphenylmethane and phthalocyanine derivatives. Textile effluents containing these dyes cause damage to the environment (Clarke and Anliker, 1980; O'Neill *et al*., 1999; Hao *et al*., 2000; Lewis, 1999)[4-7]. About 60-70% of all synthetic dyes are azo dyes and there are one or more azo bond (-N=N-) in various azo dyes[8,9]. These dyes are xenobiotic in nature and in some cases are mutagenic and carcinogenic[10, 11].

* To whom all correspondence should be addressed.
Tel.: +98-913 4031425; Fax:+98-3117866991;
E-mail: eiman.r.f@gmail.com

There are several treatment systems to degrade dyes of textile wastewater but they are very expensive[12]. Biodegradation of dyes is environmentally compatible, economical and safety method[13]. Some of microbial systems are accomplished for decolorization, operate treatment and decolorization at special qualifications of operating[3, 14]. Bacterial decolorization which is based on the break and degrade bonds between two nitrogen atoms in azo dyes[15]. Researches and studies have been accomplished about bacterial systems to decolorize various azo dyes worldwide. Different bacterial strains such as *Bacillus sp*, *Sphaerotilus natans*, *Arthrobacter* sp[16,17], *Alcaligenes faecalis*, *Commomonas acidovorans*[18], *Streptococcus faeclis*[19], *Pseudomonas aeruginosa*[20], *Shewanella* sp[21], *Rhodopseudomonas palustris*[22], *Isolate SSI*[23] are capable of decolorizing various azo dyes. The objective of this study was to compare potential capability of two single bacterial cultures, *P. aeruginosa* and *P. putida*, to decolorize four

new different azo dyes, and also evaluate the relationship between the dyes initial concentration and the decolorization percentage in order to find the optimum biodegradation ability. The degradation kinetics is essential for sophisticated design of industrial level processes and also such kinetics leads to better control of textile wastewater treatment systems. Hence the kinetic study on the biodegradation experimental data has been conducted and power-law model and its parameter has been suggested.

MATERIALS AND METHODS

Microorganisms and Dyes

Two microorganisms, *P. aeruginosa* (PTCC 1707) and *P. putida* (PTCC 1694) were obtained from Persian Type Culture Collection (PTCC, Tehran, Iran). Four different azo dyes being widely used in textile industry were subjected to biological decolorization method of this study. The powder dyes including, C.I. Acid Blue 113 (AB-113), C.I. Basic Red 46 (BR-46), C.I. Direct Blue 151 (DB-151), and C.I. Direct Brown 2 (DB-2) from UAHO company (Co UAHO, China). Different sample mixtures (Mix) containing equal concentrations of these four dyes were also prepared in distilled water.

Culture conditions

The cultivated medium in this study contained 2.5 g/L yeast extract, 4 g/L glucose, 0.5 g/L NaCl, 0.5 g/L KH_2PO_4, 0.25 g/L $MgSO_4$ and 0.25 g/L $CaCl_2$. The total volumes of experimental solutions were 100 ml containing dyes, medium and bacteria. These solutions were shaken at 150 rpm in 250 ml Erlenmeyer flasks by shaker incubator (Co. INFORS AG., Germany, Model AJ118) All experimental conditions were adjusted at pH 7.2 and C (pH-Temp. meter Co. ORION, USA, Model 240A), and also all tests were conducted on laminar hood (Co. Holten, EU, Model MS2010 1.5 GE 1LN, class 11).

EXPERIMENTAL

Decolorization assays were designed to find out degradation capacity of *P. aeruginosa* and *P. putida*, and to estimate kinetic model for better control of textile wastewater treatment systems. Each dye solution was prepared in order to make the dye initial concentration to be 0.1, 0.2, 0.5, 1, 2 (g/L). After adding medium to the prepared solution, these aqueous mediums were autoclaved for 20 min at 120 °C to inoculate bacteria. Subsequently, 0.1 g/L wet bacteria cells were added to each aqueous medium to start the biodegradation reaction. The biodegradation reaction was carried out for 48 h under shaking conditions. A sample was taken every 4 h and the color was measured.

In order to evaluate the data reproducibility and calculate the error standard deviation, all the experiments were conducted triplicate and the data mean values were presented.

Dyes analysis

The color reduction was measured using a UV–VIS spectrophotometer (Perkin Elmer, model Lambda 25, USA) at the maximum absorbance level. The wavelengths for the absorbance measurements were set at 558, 506, 554, 416, 538 nm for AB-113, BR-46, DB-151, DB-2, and the Mix, respectively. A blank sample free of dye containing bacteria cells and medium was used as the control sample. A calibration curve was prepared relating the absorbance vs. dye concentration. All the obtained calibration curves yielded a linear regression coefficient e" 0.99. The sample decolorization efficiency (DE) was expressed as follows:

$$\text{DE}\ (\%) = \frac{C_o - C}{C_o} \times 100 \qquad ...(1)$$

where is dye initial concentration and C is dye concentration of decolorized medium.

Theory

The sophisticated and economical design of industrial level treatment processes requires degradation kinetic models to create efficient condition for biodegrading textile wastewaters. The obtained experimental data were fitted to the first and second order power-law kinetic models. Using Equations (1) and (2), the slopes of linear plot of ln(/) and 1/ versus t yield the biodegradation rate constant (K) for first and second order models, respectively.

$$\ln (/) = -Kt \qquad ...(2)$$

$$1/ = Kt + 1/C_0 \qquad ....(3)$$

In the model equations, K is in and L for first order and second order models, respectively.

RESULTS AND DISCUSSION

Decolorization of Acid Blue 113

AB-113 was decolorized completely at all tested initial concentrations of 0.1, 0.2, 0.5, 1.0 and 2.0 g/L by *P. aeruginosa*. The durations of complete biodegradation were short (4 h). These results clearly indicate that higher initial dye concentration does not inhibit the microorganism activity. As shown in Fig. 1, the trend of biodegradation starts with a sharp increasing slope in the first 4 h and subsequently further processing up to 48 h does not decrease the color considerably. It seems that the dye toxicity effect does not overcome the microorganism. Therefore, it is possible to conclude that the optimum residence time of biodegradation reactor is about 4 h and no further processing is recommended. In terms of initial dye concentration, it is suggested that 2.0 g/L can be selected as the optimal value in which 100% decolorization can be achieved without losing the microorganism activity. Each data point in this research is the average of at least three independent measurements with ±1.2% standard deviation. Khehra *et al.*[24] reported complete decolorization of AB-113 at 20 mg/L concentration in 24 h by Bacillus cereus, *Pseudomonas putida*, *Pseudomonas fluorescen* and *Stenotrophomonas acidaminiphila*. Moreover, they reported that this dye has also been decolorized 99% at an initial concentration of 60 mg/l in 24 h by bacterial consortium.

Other single bacterial culture, *P. putida*, was capable of complete decolorization in the solution concentrations of 0.1 and 0.1 g/L in 8 and 32 h respectively. Applying the system for the dye initial

Table 1. Comparison of the first- and second-order kinetic constants values obtained at different initial concentrations at pH 7.2 and C for *P. aeruginosa*

Dye	C_0 (g/L)	First order kinetic model		Second-order kinetic model		Selected kinetic model	Test Duration
		$K\times10^4$ (h^{-1})	R^2	$K\times10^3$ ($Lg^{-1}\,h^{-1}$)	R^2		h
	0.1	6990	1.0	38480	1.0	first order	4
	0.2	6870	1.0	18280	1.0	first order	4
AB-113	0.5	3050	0.928	7629	0.834	first order	12
	1	1130	0.940	2544	0.841	first order	36
	2	2920	0.946	2761	0.679	first order	16
	0.1	5330	0.962	88030	0.914	first order	8
	0.2	1720	0.971	4643	0.745	first order	16
BR-46	0.5	780	0.997	459	0.923	first order	24
	1	500	0.979	107	0.989	second order	28
	2	450	0.995	45	0.965	first order	28
	0.1	2550	0.948	19080	0.899	first order	12
	0.2	1840	0.963	6689	0.797	first order	16
DR-2	0.5	920	0.940	1182	0.612	first order	32
	1	650	0.986	240	0.896	first order	32
	2	470	0.957	55	0.832	first order	32
	0.1	400	0.959	1083	0.983	second order	40
	0.2	370	0.948	240	0.973	second order	12
DB-151	0.5	110	0.971	26	0.975	second order	20
	1	90	0.974	10	0.969	first order	20
	2	-	-	-	-	-	-
	0.1	1260	0.943	12340	0.540	first order	28
	0.2	640	0.919	556	0.946	second order	16
	0.5	640	0.990	222	0.981	first order	16
Mix	1	560	0.981	90	0.958	first order	16
	2	320	0.997	26	0.984	first order	28

concentration of 0.5 and 1.0 g/L indicated 72% and 67% color elimination, respectively. *P. putida* was unable to biodegrade the dye solution with initial dye concentration of 2.0 g/L. These results clearly indicate that higher initial dye concentration causes a shortcoming in the biodegradation process. In other words, higher dye concentration creates higher toxicity which inhibits the microorganism activity. As shown in Fig. 2, a similar biodegradation trend is observed for this microorganism in comparison to *P. aeruginosa* in which a sharp increasing slope of decolorization occurs in the first 4 h and subsequently color removal becomes moderate up to 44 h. It seems that biodegradation activity ceases completely by dye toxicity effect for initial dye concentration of 2.0 g/L. Based on the obtained results, the optimum operating conditions of 12 h and 0.1 g/L may be selected for the reactor residence time and dye initial concentration. The obtained results in this investigation are comparable with Khehra *et al.*[24, 25], who achieved complete decolorization of AB-113 at 20 mg/L concentration in 24 h using different microorganism.

The obtained results show that unlike *P. aeruginosa*, the effect of initial concentration is sensible in which higher initial dye concentration inhibits the biodegradation activity for *P. putida*. As far as the microorganism decolorization activity is concerned, *P. aeruginosa* was more capable than *P.putida*. Therefore, *P. aeruginosa* is suggested for biodegradation of AB-113.

Decolorization of Basic Red 46

The results presented in Fig. 3 show that *P. aeruginosa* was capable of complete decolorization of samples with initial dye concentrations of 0.1 and 0.2 g/L. The other

Table 2. Comparison of the first- and second-order kinetic constants values obtained at different initial concentrations at pH 7.2 and 30°C for *P. putida*

Dye	C_0 (g/L)	First order kinetic model		Second-order kinetic model		Selected kinetic model	Test Duration
		$K\times10^4$ (h^{-1})	R^2	$K\times10^3$ ($Lg^{-1}\,h^{-1}$)	R^2		h
	0.1	2750	0.978	10110	0.978	first-order	8
	0.2	1040	0.956	4053	0.735	first-order	28
AB-113	0.5	250	0.944	100	0.934	first-order	40
	1	250	0.940	42	0.920	first-order	40
	2	-	-	-	-	-	-
	0.1	1380	0.921	13170	0.915	first-order	24
	0.2	1140	0.971	6129	0.911	first-order	20
BR-46	0.5	790	0.824	278	0.901	second-order	12
	1	2010	1.0	310	1.0	first-order	4
	2	1580	1.0	110	1.0	first-order	4
	0.1	1780	0.992	3958	0.987	first-order	8
	0.2	1300	0.960	4727	0.916	first-order	24
DB-2	0.5	590	0.908	342	0.964	second-order	32
	1	221	1.0	35	1.0	first-order	4
	2	180	0.942	15	0.982	second-order	44
	0.1	630	0.989	2465	0.866	first-order	36
	0.2	130	0.980	88	0.994	second-order	36
DB-151	0.5	-	-	-	-	-	-
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-
	0.1	2550	0.984	8565	0.881	first-order	8
	0.2	330	0.996	651	0.897	first-order	12
	0.5	500	0.999	977	0.907	first-order	12
Mix	1	120	1.0	967	1.0	first-order	4
	2	270	0.858	81	0.909	second-order	24

samples with initial dye concentrations of 0.5, 1.0 and 2.0 g/L were biodegraded to the maximum values of 85, 74, and 71%, respectively. Similar to AB-113, decolorization process for BR-46 has two distinct trends in which color removal takes place with a nearly sharp increasing slope up to 20 h and subsequently beyond that the color elimination becomes steady up to 44 h. These observations indicate that the optimal operating conditions may be selected 4 h and 0.1 g/L for reactor residence time and initial dye concentration, respectively. The higher toxicity level at 0.5, 1.0 and 2.0 g/L dye concentration does not allow a complete decolorization efficiency to be obtained.

Other single bacterial culture, *P. putida*, decolorized the solution concentrations of 0.1, 0.2, 0.5, 1.0 and 2.0 g/L up to nearly 55% in 4 h. Further treatment up to 24 h resulted in almost complete color removal for initial dye concentration of 0.1 and 0.2 g/L. As shown in Fig. 4, applying the system for the initial dye concentration of 0.5, 1.0 and 2.0 g/L indicated a steady color removal for further decolorization processing up to 44 h due to high toxicity level. Based on the obtained results, the optimum operating conditions of 12 h and 0.1 g/L may be selected for the reactor residence time and dye initial concentration.

As far as the microorganism decolorization activity is concerned, *P. aeruginosa* was more capable than *P. putida* for high dye initial concentration of BR-46. However, both bacteria strains demonstrated similar capability for low dye initial dye concentration of BR-46.

Decolorization of Direct Blue 151

According to Figs 5 and 6, DB-151

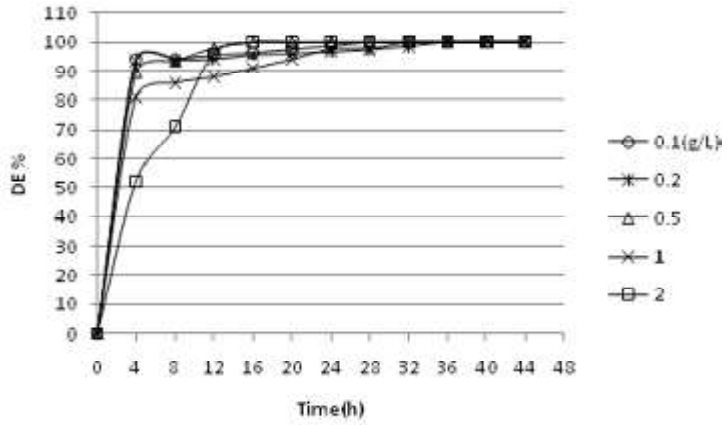

Fig. 1. Decolorization of AB-113 dye by *P. aeruginosa*

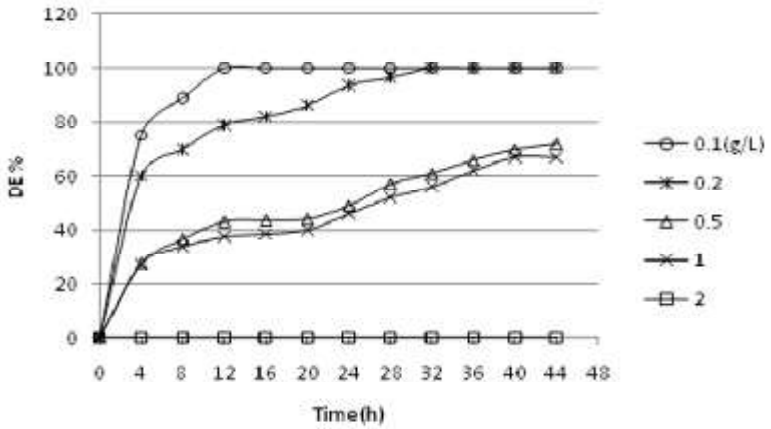

Fig. 2. Decolorization of AB-113 dye by *P. putida*

solution samples with initial dye concentrations of 0.1 and 0.2 g/L were biodegraded to the maximum values of 80 and 37%, respectively by *P. aeruginosa* and 91 and 39% respectively by *P. putida*.

Both single bacterial cultures demonstrated similar decolorization capability. These microorganisms were unable to biodegrade high initial dye concentration of DB-151 that may be attributed to dual effects of dye high molecular weight and toxicity. These two combine effects are the main cause of the inhibition ability of DB-151 in which does not allow the dye penetration through cell membranes. It is worthy to mention that the reducing biodegradability activity due to molecular weight is not dependent on the intracellular uptake of the dye[1].

Decolorization of Direct Brown 2

As the results shown in Fig. 7, the proposed treatment system by *P. aeruginosa* was capable to biodegrade all dye solution samples over 85%. DB-2 was decolorized completely in 16 and 32 h at initial concentrations of 0.1-0.2 and 0.5 g/L by *P. aeruginosa*. The dye solution with initial concentrations of 1.0 and 2.0 g/L were decolorized up to maximum DE% of 91 and 80. According to Fig. 7, the biodegradation process takes place in two stages. The trend of biodegradation for the initial dye concentrations of 0.1 and 0.2 g/L starts with a sharp increasing slope in the first 4 h and

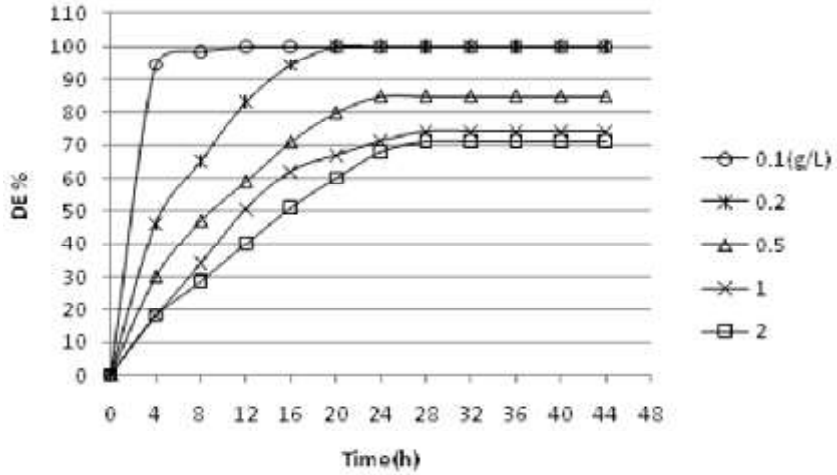

Fig. 3. Decolorization of BR-46 dye by *P. aeruginosa*

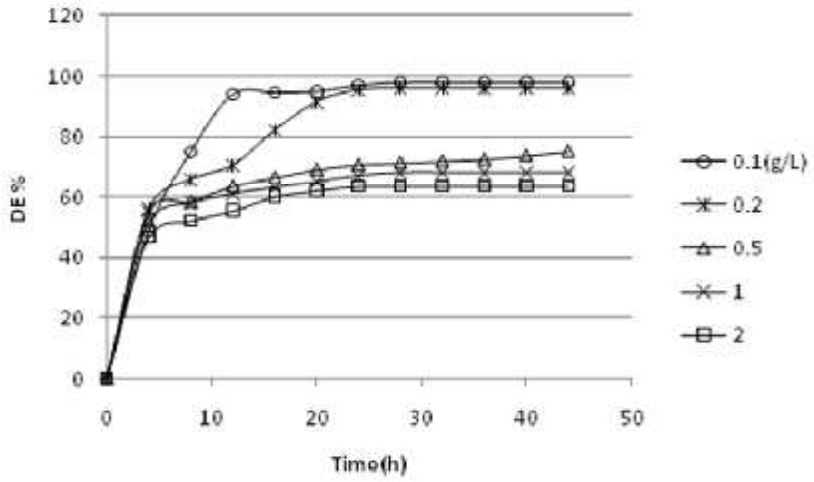

Fig. 4. Decolorization of BR-46 dye by *P. putida*

subsequently beyond that the color elimination becomes steady with a moderate slope up to 16 h. Moreover, biodegradation trend for the initial dye concentrations of 1.0 and 2.0 g/L show a moderate slop up to first 32 h and beyond that biodegradation activity ceases completely. It seems that the dye toxicity effect overcomes the microorganism capability and the biodegradation activity ceases completely beyond the 32 h reactor residence time. Therefore, it is possible to conclude that the optimum residence time of biodegradation reactor is 16 h. In terms of initial dye concentration, it is suggested that 0.2 g/L can be selected as the optimal value in which 100% decolorization can be achieved without losing the microorganism activity.

According to the obtained results in Fig. 8, *P. putida* was capable of complete decolorization in the solution concentrations of 0.1 and 0.2 g/L in 12 and 24 h respectively. The biodegradation treatment for the dye initial concentration of 0.5 and 1.0 g/L in 4 h indicated 60% color elimination and subsequently further processing up to 24 h resulted in about 80 and 70% as the final color removal respectively. The treatment process applied for the initial dye concentration of 2.0 g/L led to 28 and 60% color removal in 4 and 44 h, respectively. As shown in Fig. 8, biodegradation trend starts with a sharp increasing slope of decolorization in the first stage of treatment (4 h) and continues with a moderate slop up to 32h. Based on the obtained results, the optimum

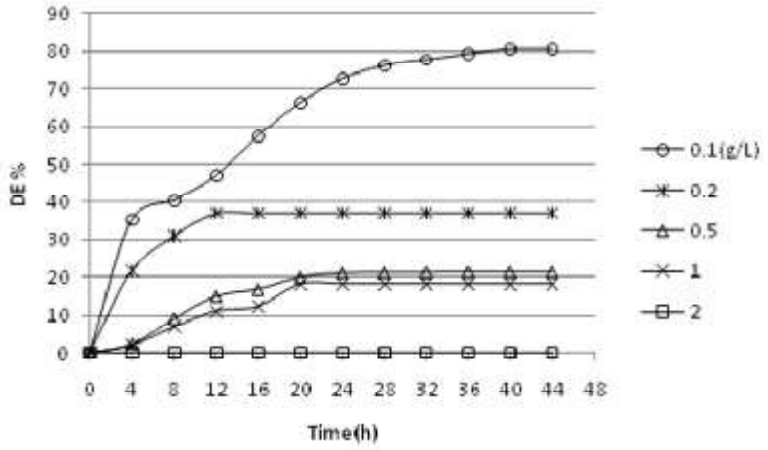

Fig. 5. Decolorization of DB-151 dye by *P. aeruginosa*

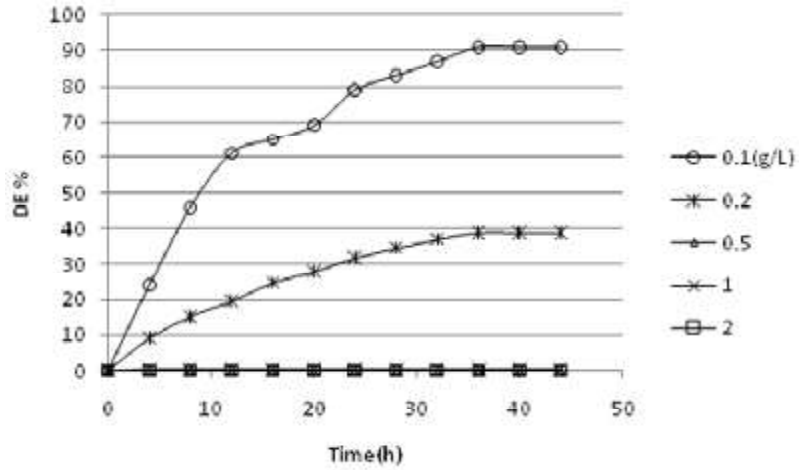

Fig. 6. Decolorization of DB-151 dye by *P. putida*

operating conditions of 12 h and 0.2 g/L may be selected for the reactor residence time and dye initial concentration.

The obtained results show that the effect of initial dye concentration is so tangible which the DE% decreased with increases in initial concentrations. As far as the microorganism decolorization activity is concerned, both *P. aeruginosa and P. putida* demonstrated very similar capability for DB-2. Similar results were reported in previous investigation for other microorganisms[26,27].

Decolorization of Mix

In order to evaluate the applicability of the proposed treatment system with the chosen microorganism to be applied for the real textile wastewater, the mixture of four dyes with equal composition was prepared. The results in Figs 9-10 demonstrate the DE% for *P. aeruginosa and P. putida,* respectively. As shown in Fig. 9, the color elimination in the first 12 h of treatment reached about 75 and 60% for the initial dye concentrations of 0.1 and 0.2-0.5 g/L, respectively. At the initial dye concentrations of 1.0 and 2.0 g/L, 12 h of treatment resulted in about 55 and 35% color decrease which is well expected for the higher initial dye concentration in contrast to low pollution loading.

Similarly, the treatment system was applied using *P. putida*. The results indicate that the overall DE% performance increased in comparison with *P. aeruginosa*. The results of Fig.

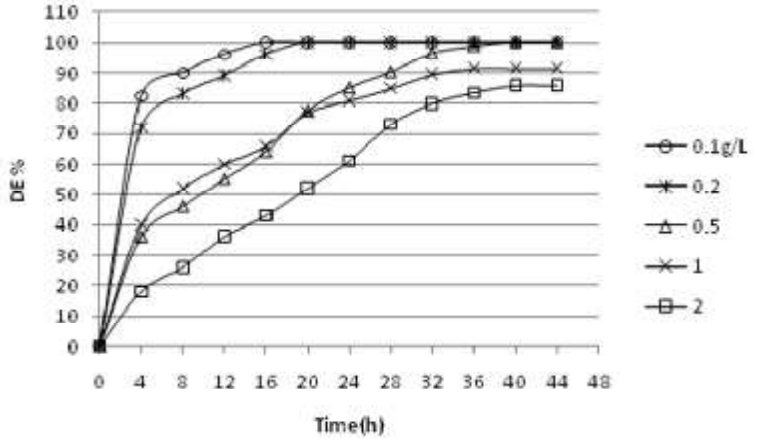

Fig. 7. Decolorization of DB-2 dye by *P. aeruginosa*

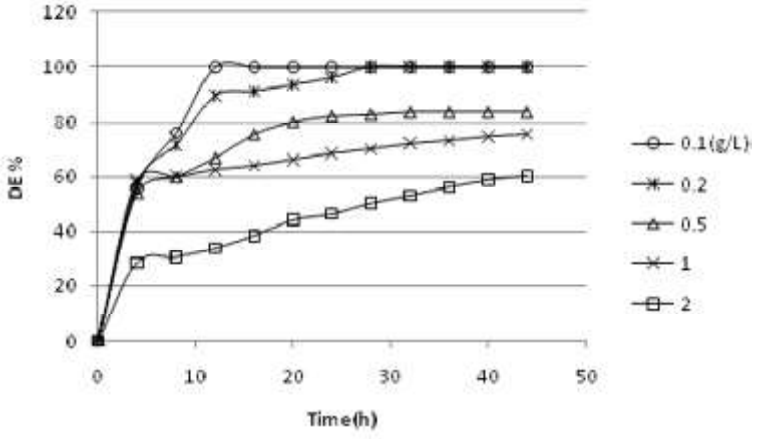

Fig. 8. Decolorization of DB-2 dye by *P. putida*

10 show that the color elimination in the first 12 h of treatment reached about 100, 86, 80 and 62% for the initial dye concentrations of 0.1, 0.2-0.5, 1.0 and 2.0 g/L, respectively. The concentration of dye substrate can affect the efficiency of biodegradation through the combination of factors including the toxicity of the dye at higher concentrations, and the ability of the enzyme to recognize the substrate efficiently at lower concentration[15]. Wuhramann *et al.*[17] reported that after an initial rapid color removal, the decolorization rate decreased, however, Dubin and Wright reported the absence of any effect of dye concentration on reduction rate that is compatible for non-enzymatic color removal mechanism[28].

The obtained experimental results indicate that the proposed treatment system is a viable method for the textile industries. The initial dye concentration of 0.2-0.5 g/L and residence time of 4 h utilizing *P. putida* are suggested as the optimum conditions in which over 75% decolorization can be achieved without losing the microorganism activity for the mixed synthetic wastewater treatment.

Effect of dyes structures and dyes molecular weights

The variation of decolorization efficiency with different initial dye concentration and structure for these bacteria strains are depicted in Figs 11 and 12. Resistance of azo dyes to removal by

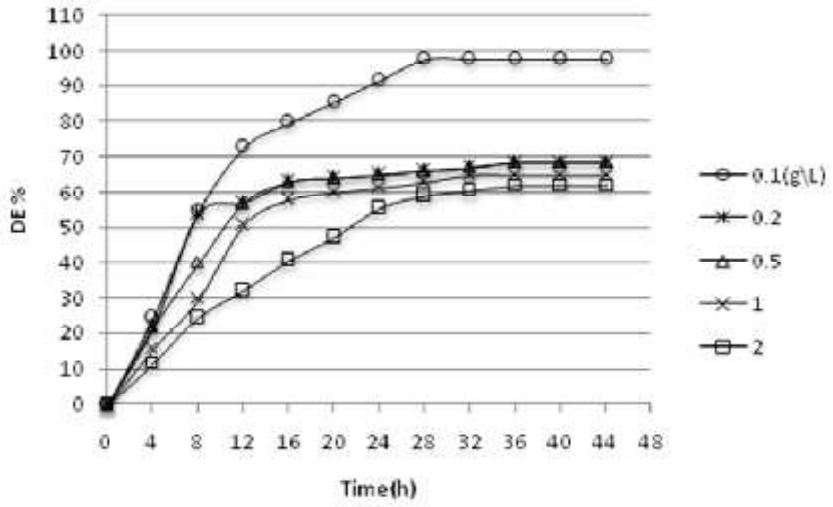

Fig. 9. Decolorization of Mix dye by *P. aeruginosa*

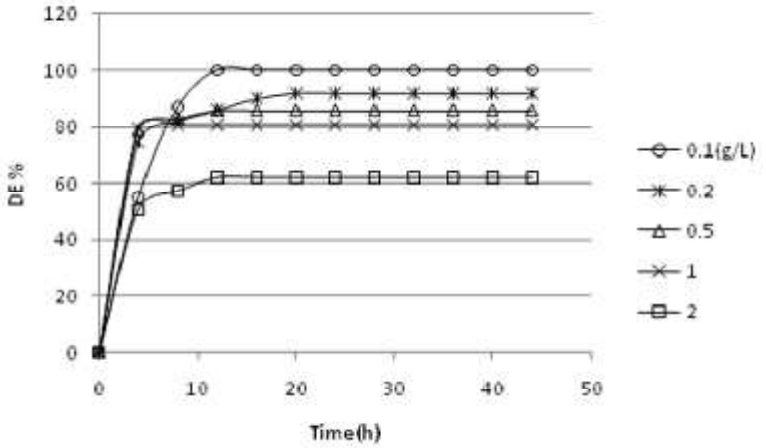

Fig. 10. Decolorization of Mix dye by *P. putida*

bacterial cell is various[29]. Dyes with simple structures and lower molecular weight show higher rates of color reduction; whereas, higher molecular weight dyes exhibit lower color removal efficiency[30], because they are improbable to pass through cell membrane[2]. In addition, color removal is related to the number of azo bonds in the dye molecule. Monoazo dyes biodegrade faster than the diazo and triazo dyes[31]. The number of azo bonds and molecular weight of azo dyes used in this study are as follows: DB-151 (diazo; 773.7), AB-113 (diazo; 681.65), DB-2 (diazo; 627.51), and BR-46 (monoazo; 403.319 g/mole). The DB-151, with highest molecule weight and being diazo, showed lowest dye removal efficiency in this study and therefore this result confirmed the aforementioned relationship between color elimination with the dye structure. In contrast, BR-46 with lowest molecular weight showed acceptable dye removal efficiency as a monoazo dye. Further research is needed to clarify the detailed molecular mechanism of decolorization by these bacteria cells.

Biodegradation kinetics

To determine the reaction rate of dye reduction in the biodegradation CSBR (Continuous Stirred Batch Reactor), periodically a 5 ml sample was collected and after pH adjustment to 7.2, the color reduction was measured, the color removal measurement was pursued up to time the microorganism was workable noticeably. For the

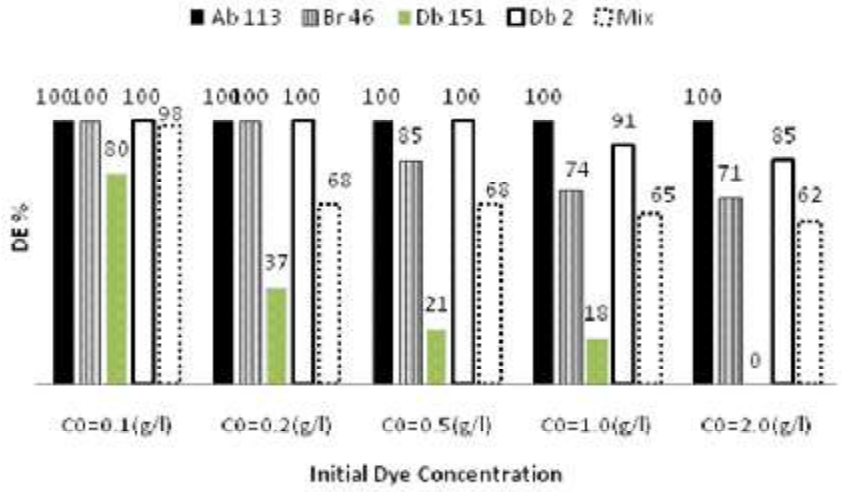

Fig. 11. *P. aeruginosa* biodegradation of five different dye solution

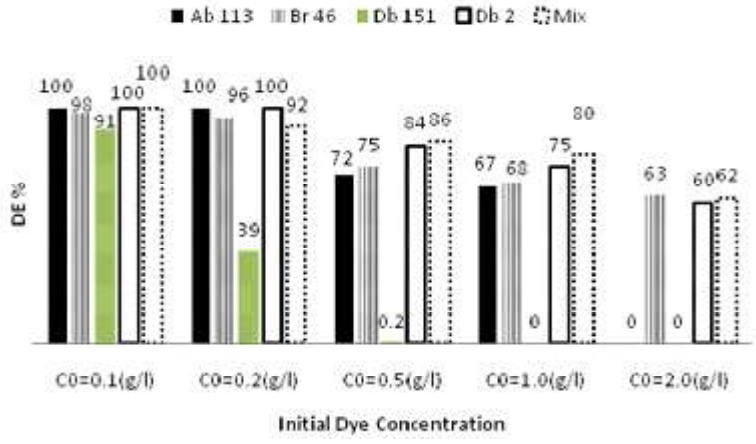

Fig. 12. *P. putida* biodegradation of five different dye solution

most of dye solution measurement was carried out for first 8 h. The color reduction versus time data was obtained and different reaction orders were investigated. The tests for the first-order and second-order reaction in terms of dye removal are shown in Tables 1-2. Since the experimental data fall on a reasonably straight line with acceptable R^2 values for the least squares, it can be concluded that the rate of color removal follows the first-order or second-order with respect to dye concentration. Applying the linear least square technique, the reaction rate constant (K) was evaluated as the slope of the obtained straight line. The calculated values for the reaction rate constants using two different microorganisms at various dye concentrations are shown in Table 1 (*P. aeruginosa*) and Table 2 (*P. putida*). Similar results and kinetic models were reported for biodegradation of hybrid textile wastewater and G Black RL[32,33].

The results of this study show that decolorization reaction takes place with higher rate constants at low initial dye concentrations utilizing two bacteria strains. The obtained results indicate that the decolorization is faster for AB-113 in comparison with other dyes. Moreover, *P. aeruginosa* decolorized BR-46, DB-151 and DB-2 faster than other single bacterial culture. Whereas, P. *putida* degraded AB-113 and Mix more quickly.

CONCLUSIONS

The biodegradation activity and effectiveness of two microorganisms, *P. aeruginosa* and *P. putida*, was investigated for 4 different azo dyes and the mixture of them. The kinetic models of color elimination were studied by evaluating reaction order and rate constant. The first and second order rate equations are compatible for the biodegradation reaction of the two used microorganisms. The higher calculated rate constants of first and second order were observed at low dye concentrations. The results indicate that the color removal is a strong function of initial dye concentration, dye molecular weight and number of azo bonds. Among different azo dyes studied, AB-113 was the easiest dye to biodegrade by *P. aeruginosa*. *P.putida* showed better decolorization efficiency for the mixture solution in contrast to *P. aeruginosa*. As far as the optimum operating conditions are concerned, the reactor residence time (h) and initial dye concentration were investigated for both single bacterial cultures. Overall, the applied treatment system is viable method for removal of chromophores from the disposed effluents by textile industries.

ACKNOWLEDGEMENTS

The financial support is provided for this project by Isfahan University of Technology (IUT) and Isfahan Science and Technology Town (ISTT) are gratefully acknowledged. This is to thank sincere technical help and cooperation by Shayan Andish Company.

REFERENCES

1. Fu, Y., Viraraghavan, T. Fungal decolourization of dye wastewater: a review. *Bioresource Technol*., 2001; **79**: 251–262.
2. Robinson, T., McMullan, G., Marchant, R., Nigam, P. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Bioresource Technol.,* 2001; **77**: 247–255.
3. McMullan, G., Meehan, C., Conneely, A., Kirby, N., Robinson, T., Nigam, P., Banat, I.M., Marchant, R., Smyth, W.F. Microbial decolourisation and degradation of textile dyes. *Appl. Microbiol. Biot.,* 2001; **56**: 81-87.
4. Clarke, E.A., Anliker, R. Organic dyes and pigments. Hand-book of environmental chemistry, anthropogenic compounds, Part A, vol. 3. New York: Springer., 1980; 181–215.
5. O'Neill, C., Hawkes, F.R., Hawkes, D.L., Lourenco, N.D., Pinheiro, H.M., Del´ee, W. Colour in textile effluents—sources, measurement, discharge consents and simulation: a review. *J. Chem. Technol. Biot.,* 1999; **74**: 1009–1018.
6. Hao, O.J., Kim, H., Chiang, P.C. Decolorization of wastewater. Crit. Rev. Env. Sci. Tec., 2000; 30 (4): 449–505.
7. Lewis, D.M. Coloration for the next century. *Rev. Prog. Color Relat Topics*., 1999; **29**: 23-28.
8. Carliell, C.M., Barclay, S.J., Naidoo, N., Buckley, C.A., Mulholland, D.A., Senior, E. Microbial decolourisation of a reactive azo dye under anaerobic conditions. Water SA., 1995; **21**(1): 61-69.
9. Banat, I.M., Nigam, P., Singh, D., Marchant, R. Microbial decolorization of textile-dyecontaining effluents: a review. *Bioresource*

Technol., 1996; **58**: 217-227.
10. Daneshvar, N., Ayazloo, M., Khatae, AR., Pourhassan, M. Biological decolorization of dye solution containing malachite green by microalgae Cosmarium sp. Bioresource Technology., 2007; 98: 1176-1182.
11. Dafale, N., Agrawal, L., Kapley, A., Meshram, S., Purohit, H., Wate, S. Selection of indicator bacteria based on screening of 16S rDNA metagenomic library from a two-stage anoxic–oxic bioreactor system degrading azo dyes. *Bioresource Technology.,* 2010; **101**: 476–484.
12. Coughlin, M.F., Kinkle, B.K., Tepper, A., Bishop, P.L. Characterization of aerobic azo dye degrading bacteria and their activity in biofilms. Water SA., 1997; **36**: 215–220.
13. Moosvi, S., Keharia, H., Madamwar, D. Decolourization of Textile dye reactive violet 5 by a newly isolated bacterial consortium RVM 11.1. *World J. Microb. Biot.,* 2005; **21**: 667–672.
14. Chen, K.C., Wu, J.Y., Liou, D.J., Hwang, S.C.J. Decolorization of textile dyes by newly isolated bacterial strains. *J. Biotechnol.,* 2003; **10**: 57-68.
15. Pearce, C.I., Lloyd,J.R., Guthrie, J.T. The removal of colour from textile wastewater using whole bacterial cells: a review. Dyes and Pigments., 2003; **58**: 179–196.
16. Zissi, U., Lyberatos, G. Azo-dye biodegradation under anoxic conditions. Water SA, 1996; **34**(5-6): 495–500.
17. Wuhrmann, K., Mechsner, K., Kappeler, T. Investigations on rate determining factors in the microbial reduction of azo dyes. *Eur. J. Appl. Microbiol. Biot*., 1980; **9**: 325-338.
18. Nigam, P., Banat, I.M., Singh, D., Marchant, R. Microbial process for the decolorization of textile effluent containing azo, diazo and reactive dyes. *Process Biochem.,* 1996; **31**(5): 435–442.
19. Gingell, R., Walker, R. Mechanisms of azo reduction by *Streptococcus faecalis*. II. The role of soluble flavins. Xenobiotica.,1971;1 (3): 231–239.
20. Bhatt, N., Patel, K.C., Keharia, H., Madamwar, D. Decolorization of diazo-dye Reactive Blue 172 by *Pseudomonas aeruginosa* NBAR12. *J. Basic Microbiol.,* 2005; **45**(6): 407-418.
21. Khalid, A., Arshad, M., Crowley, D.E. Decolorization of azo dyes by *Shewanella sp*. under saline conditions, *Appl. Microbiol. Biot*., 2008; **79**: 1053-1059.
22. Liu, G-F., Zhou, J-T., Wang, J., Song, Z-Y., Qv, Y-Y. Bacterial decolorization of azo dyes by *Rhodopseudomonas palustris*. *World J. Microb. Biot.,* 2006; **22**: 1069 - 1074.
23. Mahmood, S., Arshad, M., Khalid, A., Nazli, Z.H., Mahmood, T. Isolation and screening of azo dye decolorizing bacterial isolates from dye-contaminated textile wastewater. *Soil. Environ*., 2011; **30**(1): 7-12
24. Khehra, M.S., Saini, H.S., Sharma, D.K., Chadha, B.S., Chimni, S.S. Comparative studies on potential of consortium and constituent pure bacterial isolates to decolorize azo dyes. Water Res., 2005; **39**: 5135–5141.
25. Khehra, M.S., Saini, H.S., Sharma, D.K., Chadha, B.S., Chimni, S.S. Decolorization of various azo dyes by bacterial consortium. *Dyes and Pigments*., 2005; **67**: 55-61.
26. Hsueh, C.C., Chen, B.Y. Comparative study on reaction selectivity of azo dye decolorization by *Pseudomonas luteola*. J. Hazard. Mater., 2007; **141**: 842–849.
27. Gopinath, K.P., Sahib, H., Muthukumar, K., Velan, M. Improved biodegradation of Congored by using *Bacillus sp*. Bioresource Technol., 2009; **100**: 670-675
28. Dubin, P., Wright, K.L. Reduction of azo food dyes in cultures of *Proteus vulgaris*. Xenobiotica., 1975; **5**(9): 563–571.
29. Bras, R., Ferra, I.A., Pinheiro, H.M., Goncalves, I.C. Batch tests for assessing decolourisation of azo dyes by methanogenic and mixed cultures. *J. Biotechnol.,* 2001; **89**: 155–162.
30. Sani, R.K., Banerjee, U.C. Decolorization of triphenyl-methane dyes and textile and dye-stuff effluent by *Kurthia sp*. Enzyme Microb. Tech., 1999; **24**: 433–437.
31. Hu, T-L. Kinetics of azoreductase and assessment of toxicity of metabolic products from azo dyes by *Pseudomonas luteola*. Water SA., 2001; **43**(2): 261–269.
32. Sandhya, S., Sarayu, K., Swaminathan, K. Determination of kinetic constants of hybrid textile wastewater treatment system. *Bioresource Technol*., 2008; **99**: 5793–5797.
33. Aksu, Z., Karabayýr, G. Comparison of biosorption properties of different kinds of fungi for the removal of Gryfalan Black RL metal-complex dye. *Bioresource Technol.,* 2008; 99: 7730-7741.