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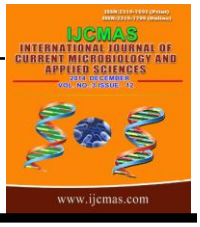

Original Research Article

Bioremediation of Textile Wastewater Dyes using Local Bacterial Isolates

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Keywords

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A B S T R A C T

Dye contaminated wastewater and soil samples were collected from a textile factory at 10th of Ramadan industrial city, Egypt. These samples were studied in order to isolate some bacterial species which has the capability to decolorize textile dyes. Acid Red (AR) 151, Orange (Or) II, Sulfur Black (Sb) and Drimarene Blue (Db) K2RL dyes were used in the study. The most efficient bacterial isolates (high decolorization zone) were identified using Biolog® Gen III system and they were *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Bacillus cereus*. Decolorization abilities of isolates were studied and optimization of physicochemical parameters (agitated versus static condition, effect of pH, effect of dye concentrations and incubation periods) was also examined. Ability of bacterial isolates for decolorization of textile wastewater effluents was also examined and toxicity of final effluent was measured using Microtox® analyzer 500. Results showed that, static incubation conditions showed higher decolorization ratios than agitated incubation, optimum pH for decolorization was 7.0, highest decolorization observed at dye concentration of 600 mgL^{-1} and by increasing the incubation period, a gradual increase in decolorization was observed. Finally, local bacterial isolates were able to decolorize textile wastewater effluents

Introduction

The fast growth in urbanization and industrialization results in the discharge of huge amounts of pollutants and wastes into the environment (Senan and Abraham, 2004). Synthetic dyes are widely used in many industries including paper printing, textile dyeing, pharmaceutical, color photography, cosmetics, food and other industries. During textile dyeing process, the class of dye used is responsible for the amount of dye lost in effluent which vary from 2% and 50% loss in case of using basic dyes and reactive dyes, respectively. At least, 20% of these losses reach the environment through wastewater treatment plants effluents (Kirk and Othmer, 1993; McMullan *et al.*, 2001; Sheth and Dave, 2009).

Azo dyes are considered as one of the major dyes that affect the environment because of their bio-recalcitrance, color and potential

toxicity to human and animals. The textile and dyeing effluents are characterized by high COD, BOD, color, pH and presence of metals. As a result, it is not easy to treat these effluents using the common physicochemical treatment methods. In addition, these physicochemical treatment methods have some limitations such as production of large amounts of sludge, expensive and emission of toxic substances (Johnson, 1978; Levine, 1991; Senan and Abraham, 2004). Also, azo dyes can contaminate drinking water supplies and enzymatically degraded inside the human digestive system and producing carcinogenic substances (Kirk and Othmer, 1993; Sheth and Dave, 2009).

There are three main treatment methods that used for dyes effluent treatment which are chemical, physical and biological.The chemical and physical methods include flocculation, coagulation, adsorption, incineration, irradiation and ultra-filtration. These chemical and physical methods have disadvantages such as requirement of high capital costs and difficulty in handling (Papic´ *et al.,* 2000; Chakraborty *et al.,* 2003; Georgiou *et al.,* 2003; Patil *et al.,* 2008).

The biological treatment methods which depend on the ability of microorganisms to decolorize dyes have received much attention (Verma and Madamwar, 2003; Moosvi *et al.,* 2005). Previous studies showed the ability of a wide range of microorganisms such as bacteria and white rot fungi to decolorize dyes (Asgher *et al.,* 2006; Dave and Dave, 2009).

The main aim of the present study is the isolation and identification of some bacterial species (from naturally dye contaminated wastewater and soil samples) which have the ability to decolorize and degrade (bioremediation) some azo dyes used in textile industry. In addition, optimization of bacterial isolates was carried out for maximum bioremediation ability.

Materials and Methods

Samples and sampling

Wastewater samples (*n*=10) from textile industry effluent and soil samples (*n*=10) naturally contaminated with dyes were collected from textile factory at 10th of Ramadan industrial city, Egypt. All samples were collected in sterile 1L polypropylene bottles, kept at 4°C and transferred to laboratory during 24h.

Dyes and media

Four different dyes were used for studying bioremediation process including Acid Red (AR) 151, Orange (Or) II, Sulfur Black (Sb) and Drimarene Blue (Db) K2RL which used by the textile factory. Nutrient broth and agar media were used for isolation and preservation of bacterial isolates. All chemicals and media used in the study were purchased from Sigma chemicals Co. (St. Louis, USA).

Isolation of decolorizing bacteria

Separately, 1 mL of each wastewater sample and 1.0 g of each soil sample was inoculated in 100 ml sterile distilled water in 250 mL Erlenmeyer flask. Inoculated flasks were incubated in shaker incubator at 120 rpm and 35°C for 24h. The obtained suspensions were diluted 10 times with sterile saline solution and 0.1 mL of each dilution was streaked on four nutrient agar plates, each plate containing 300 ppm of single studied dye and incubated at 35°C for 24h and decolorization zones were measured after incubation period. Colony showing the

largest decolorization zone in each plate was picked up and preserved on nutrient agar slants for further studies.

Identification of decolorizing bacteria

The picked up bacterial isolates were identified using Biolog Gen III (Biolog® Inc., USA) identification system.

Inoculums preparation

A cell suspension (~ 107 cfu mL^{-1}) from each preserved slant was inoculated in nutrient broth (NB) containing 300 ppm of each dye separately and incubated at 35°C. On 90–95% decolorization of the added dye, 20% (v/v) actively growing culture was used as inoculum for next cycle. The culture suspension in the decolorized broth was standardized. The cells were harvested by centrifugation at 6,000g for 25 min (REMI C-24 centrifuge) and the inoculums were standardized with respect to optical density of 0.8 at 660 nm, using UV-Visible spectrophotometer (UV 2100, Shimadzu, Japan).

Decolorization study and quantification

For the decolorization study, 10 mL of the uninoculated and inoculated broths were centrifuged at 10,000 rpm for 15 min and the supernatants were checked for decolorization at 520 nm. Dye concentration of the decolorized broth was quantified by comparing its absorbance with the absorbance of known concentrations (initial absorbance) of studied dyes (Acid Red (AR) 151, Orange (Or) II, Sulfur Black (Sb) and Drimarene Blue (Db) K2RL) and this was used to calculate the dye removal rate (mg L^{-1} h^{-1}) and expressed as percentage of decolorization (Kumar *et al.,* 1997; Chen *et al.,* 1999). Percent of decolorization was calculated as:

$$\text{Decolorization (\%)} = \frac{I-F}{I} \times 100$$

Where I = initial absorbance and F = absorbance of decolorized medium.

Optimization of physicochemical parameters

The influence of agitated versus static condition (120 rpm), effect of different pH values (5-10), effect of different dye concentrations (100-1000 mgL^{-1}) and incubation periods (1-36 h) were studied in terms of decolorization of dye using the spectrophotometer. If otherwise mentioned, experiments were performed using 20% (v/v) actively growing cultures as inocula containing 107 $cfumL^{-1}$, 300 ppm dye concentration, and 7.0 ± 0.2 pH, at 35°C under static condition. All the experiments were performed in triplicates.

Decolorization of textile wastewater effluent

Wastewater samples were collected from a textile factory at 10th of Ramadan industrial city, Egypt. Samples were collected from production line, where each of studied dyes were used in dying processes. Collected wastewater samples were first filtered twice using membrane filtration technique (pore size 0.45µm, Whatman®) for removing debris and microorganisms from the samples. Dye concentrations were quantified by comparing it with the color development of known concentrations of studied dyes. COD (chemical oxygen demand) and pH were measured by standard procedure (APHA, 2005). Each 200 mL of these effluents were inoculated with 20% (v/v) developed active bacterial culture, in 500 mL Erlenmeyer flasks then incubated at 35°C for decolorization study.

Toxicity assay

The bioremediated (decolorized/ biodegraded) products were tested for their toxic effect using Microtox® analyzer model 500 (Strategic Diagnostics Inc., USA).

Results and Discussion

Screening and identification

Eight bacterial isolates were isolated from wastewater samples (4 isolates) and soil samples (4 isolates) which showed higher decolorization zones. All eight isolates were resistant to 300 ppm of studied dyes (Acid Red (AR) 151, Orange (Or) II, Sulfur Black (Sb) and Drimarene Blue (Db) K2RL) indicating that these isolates are naturally adapted as they were from dye-contaminated samples.

Similar results were reported by Khehra *et al.,* (2005) and Sheth and Dave, (2009). The eight isolates were identified using Biolog Gen III system (Biolog® Inc., USA) and they were found *Pseudomonas aeruginosa* (4 isolates), *Pseudomonas putida* (2 isolates) and *Bacillus cereus* (2 isolates). Some previous studies reported the ability of *Ps. aeruginosa* (Sheth and Dave, 2009), *Ps. putida* (Mansour *et al.,* 2013) and *B. cereus* (Liao *et al.,* 2013) for decolorization of textile dyes.

Influence of agitated versus static condition

The actively growing inocula of *Ps. aeruginosa*, *Ps. putida* and *B. cereus* required only 5-6 h for 90-94% decolorization of 300 ppm of Acid Red (AR) 151, Orange (Or) II, Sulfur Black (Sb) and Drimarene Blue (Db) K2RL dyes under static condition. The agitation incubation conditions of flasks showed no considerable decolorization of dyes, suggesting that better dyes decolorization was occurred under facultative anaerobic conditions, which may be due to the presence of azo reductase (Sandhya *et al.,* 2008). Chang and Lin (2000) have that azo reductase is inhibited primarily by the presence of oxygen due to competition in the oxidation of reduced electron carriers with either oxygen or azo groups receptor.

Effect of different pH values

pH concentration showed deep effects on the different biological activities of studied bacterial species. The optimum pH for dyes decolorization ranged between 6.5 and 7.5 as the decolorization process was achieved in less than 7 h. The maximum decolorization was occurred at pH 7.0 (Figure 1).

The same results were reported by Bhatt *et al.,* (2005) as they have found that optimum decolorization rate of diazo-dye Reactive Blue 172 by *Pseudomonas aeruginosa* NBAR12 was observed at pH 7.0.

Similarly, Tripathi and Srivastava (2011) reported that maximum decolorization of Acid Orange 10 azo dye was observed at pH 7.0 by using both *Pseudomonas putida* and *Bacillus cereus*.

Effect of different incubation periods

The effect of incubation period (1-36 h) on decolorization of studied dyes by using *Ps. aeruginosa*, *Ps. putida* and *B. cereus* was investigated. Figure (2) showed that there was a gradual increase in decolorization rate of the four studied dyes by increasing the incubation period. This may be attributed to the active growth of used cultures in the study (Sheth and Dave, 2009; Alalewi, and Jiang, 2012).

Fig.1 Effect of different pH values on dye decolorization using (a) *Ps. aeruginosa*, (b) *Ps. putida* and (c) *B. cereus*

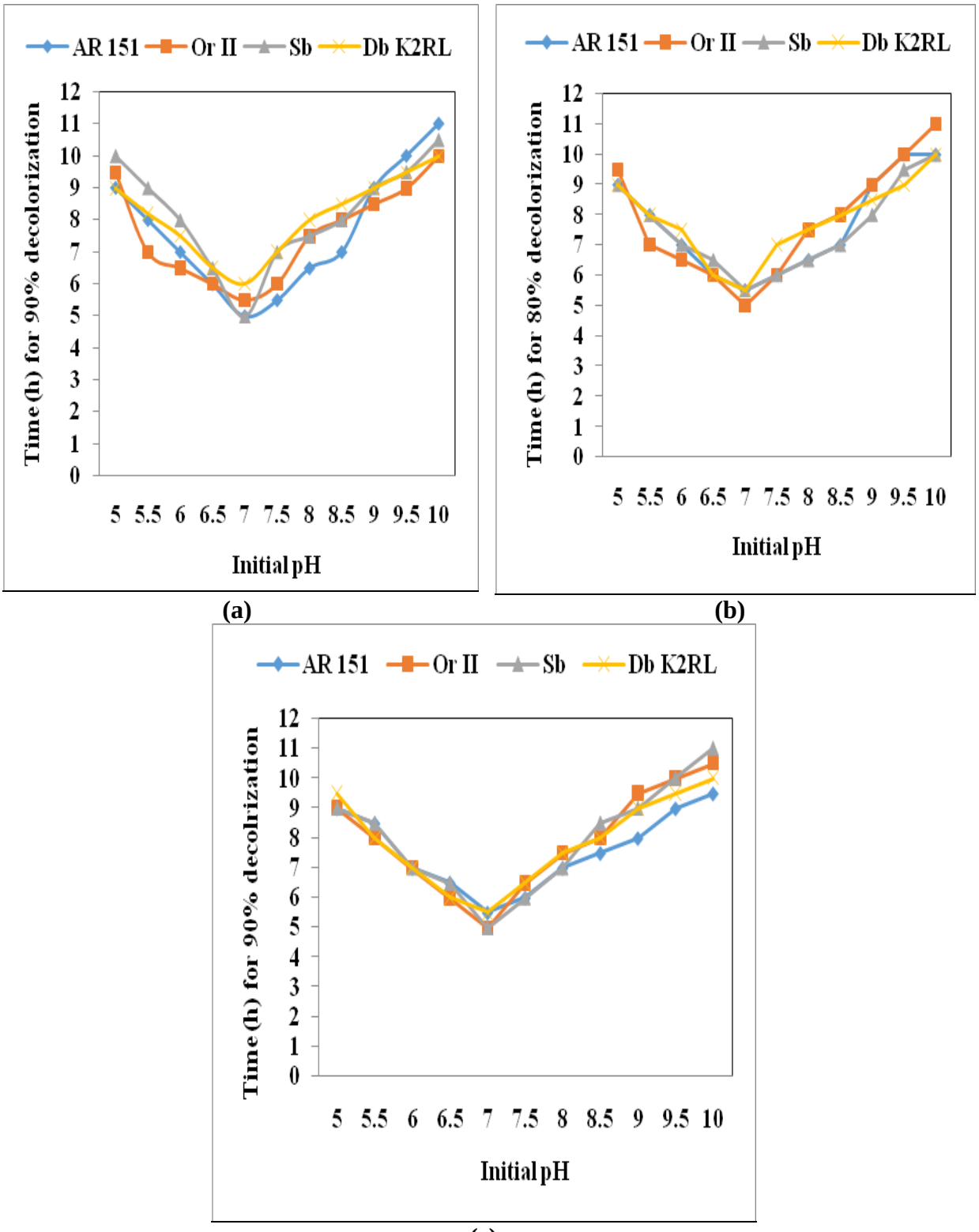
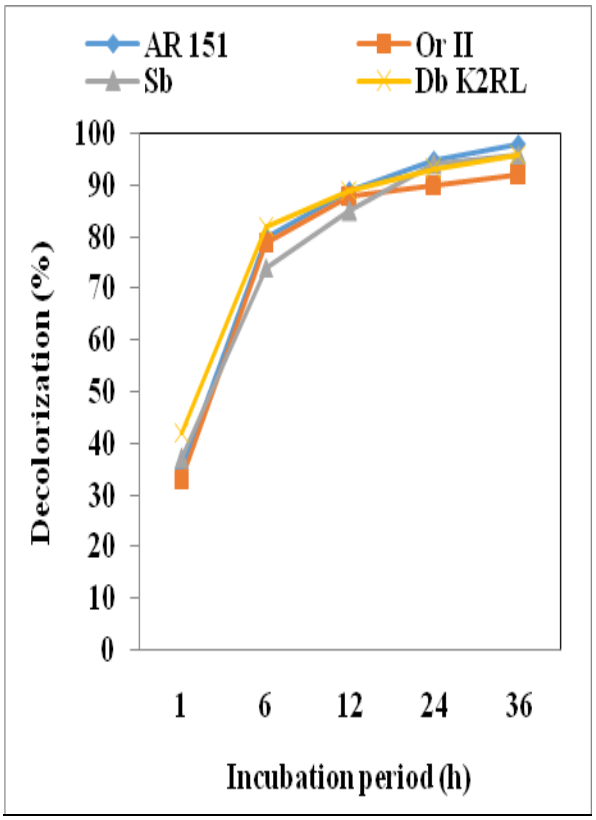
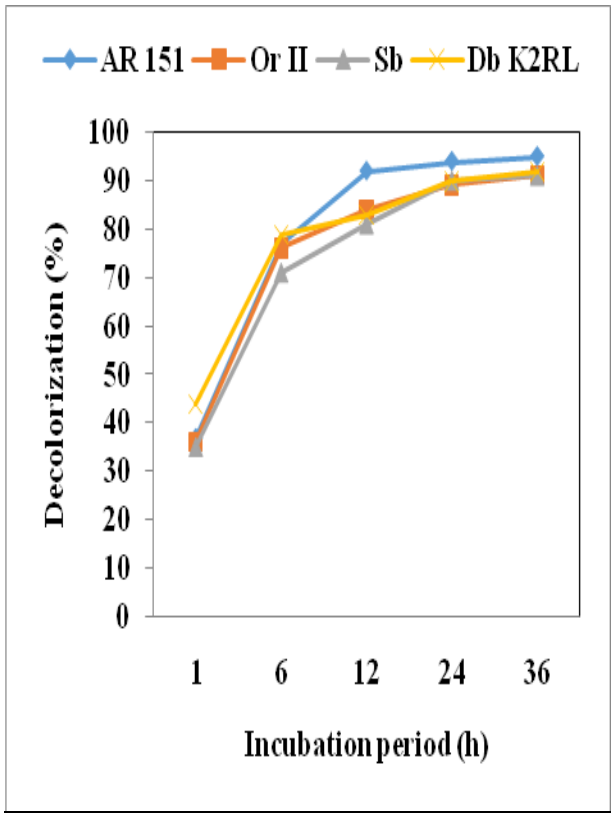


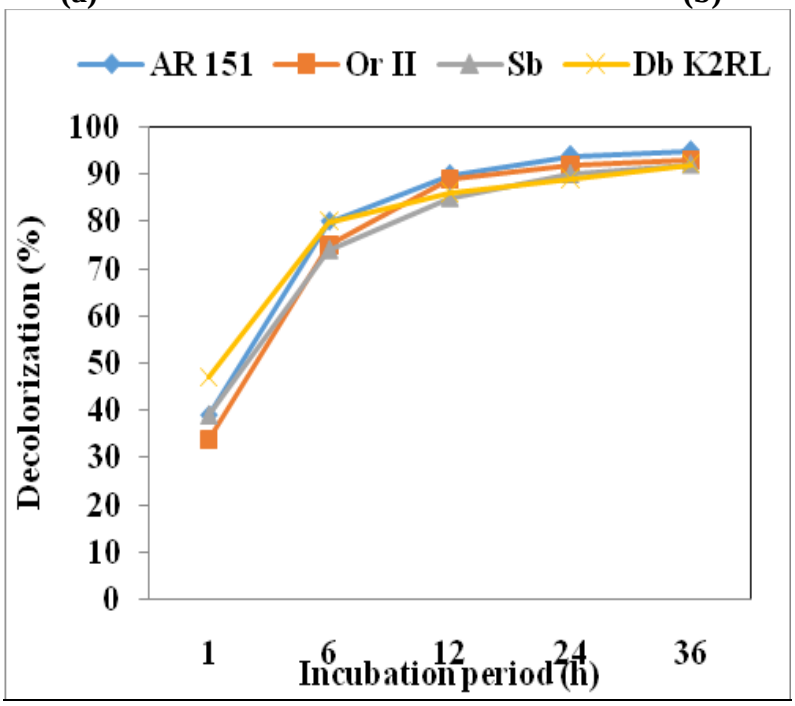
Fig.2 Effect of different incubation periods on dye decolorization using (a) *Ps. aeruginosa*, (b) *Ps. putida* and (c) *B. cereus*



(a)

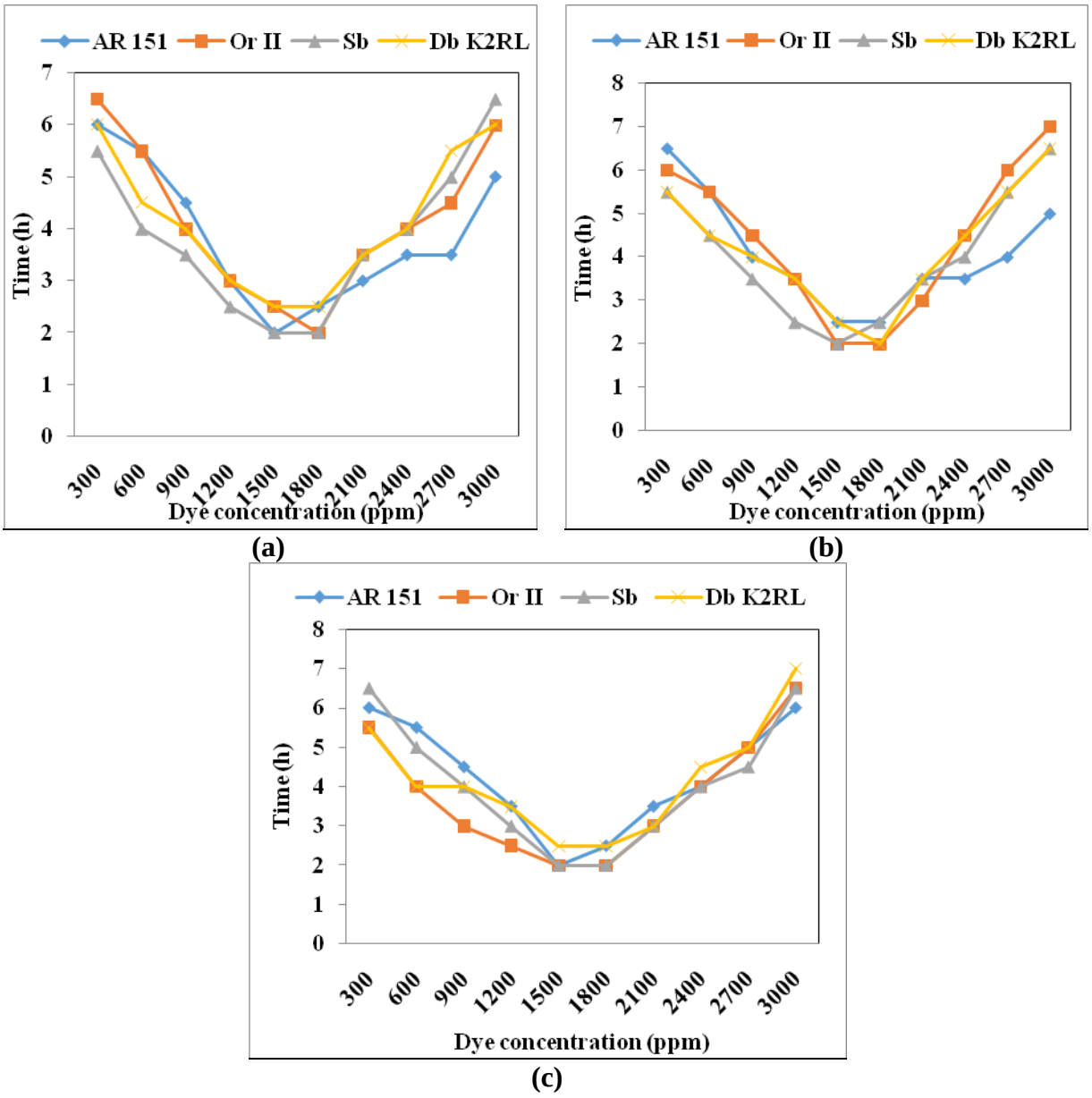


(b)



(c)

Fig.3 Effect of different dye concentrations on dye decolorization using (a) *Ps. aeruginosa*, (b) *Ps. putida* and (c) *B. cereus*



Effect of different concentrations of dyes

The influence of dye concentration (100-1000 mg L^{-1}) on the decolorization ability of *Ps. aeruginosa*, *Ps. putida* and *B. cereus* was investigated (Figure 3). The obtained results showed that the required time for dyes decolorization was directly proportional to the dye concentration. The maximum decolorization rate was obtained at 600 mg L^{-1} dye concentration with 85.5, 89.0 and 90.2% decolorization for *Ps. aeruginosa*, *Ps. putida* and *B. cereus*, respectively. A gradual inhibition of decolorization ability of used bacteria was observed at dye concentration of 800 mg L^{-1} and above. Similar results were reported by Sheth and Dave (2009) for *Ps. aeruginosa*

and by Tripathi and Srivastava (2011) for both *Ps. putida* and *B. cereus*.

Decolorization of textile wastewater effluents

The present study was carried out to develop a biological treatment process for textile wastewater effluents through studying the decolorization ability of some local bacterial isolates. Wastewater effluent from textile industry with 8000, 9500, 10000 and 8500 ppm of Acid Red (AR) 151, Orange (Or) II, Sulfur Black (Sb) and Drimarene Blue (Db) K2RL, respectively, showed 90, 86, 84 and 92% decolorization, respectively and 84, 88, 91 and 89% reduction in the COD, respectively during 48 h contact time.

Toxicity assay

The toxicity of final products in flasks after finishing the bioremediation process (decolorized/degraded) of studied dyes using bacterial isolates were measured using Microtox® analyzer model 500 (Strategic Diagnostics Inc., USA). The Microtox® photometer measures the light levels emitted by naturally luminescent *Vibrio fischeri*before and after addition of the sample, and the reduction in light output is a measure of the toxicity of the sample. The obtained results showed that all treated samples were non-toxic. Similar reports by Mali *et al.,* (2000) for non-toxicity of biologically treated Brilliant green, Malachite green, Methylene blue, Fast green and Congo red dyes.

From the obtained results, it can be concluded that: Locally isolated strains *Ps. aeruginosa*, *Ps. putida* and *B. cereus* showed high decolorization and degradation activities of textile dyes and wastewater effluents. Static incubation conditions showed higher decolorization ratios than agitated incubation. Optimum pH for maximum biodegradation was 7.0. Time required for decolorization of studied dyes was directly proportional to the concentration of dye. Highest decolorization observed at dye concentration of 600 mg L^{-1}. By increasing the incubation period, a gradual increase in decolorization was observed. Locally isolates were able to decolorize textile wastewater effluents with non-toxic final effluent.

References

Alalewi, A.; Jiang, C. (2012). Bacterial Influence on Textile Wastewater Decolorization. *J. Environ. Prot.*, 3: 889-901.

APHA (2005). American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 21st edn., Washington, D.C.

Asgher, M.; Shah, S.A.H.; Ali, M.; Legge, R.L. (2006). Decolorization of some reactive textile dyes by white rot fungi isolated in Pakistan. *World J. Microbiol. Biotechnol.*, 22: 89–93.

Ben Mansour, H.; Ridha, M.; Kamel, G.; Leila, C. (2011). Decolorization of Textile Wastewater by Pseudomonas putida*: Toxicity Assessment.* Environ. Eng. Sci., *28(7): 489-495.*

Chang, J.S.; Lin, Y.C. (2000). Fed-batch bioreactor strategies for microbial decolorization of azo dye using a *Pseudomonas luteola*strain. *Biotechnol. Progress,* 16: 979–985.

Chen, K.C.; Huang, W.T.; Wu, J.Y.; Houng, J.Y. (1999). Microbial decolorization of azo dyes by *Proteus mirabilis*. *J. Ind. Microbiol. Biotechnol.,* 23(1): 686–690.

Clarke, G.M. (1994). Statistics and experimental design—an introduction for biologists and biochemists, 3rd

edn. Edward Arnold, London, pp 164–174.

Dave, S.R.; Dave, R.H. (2009). Isolation and characterization of *Bacillus thuringiensis* for Acid red 119 dye decolorization. *Biresour. Technol.,* 100: 249–253.

Kumar, V.; Wati, L.; FitzGibbon, F.; Nigam, P.; Banat, I.M.; Singh, D.; Marchant, R. (1997). Bioremediation and decolorization of anaerobically digested distillery spent wash. *Biotechnol. Lett.,* 19(4): 311–314.

Liao, C.S.; Hung, C.H.; Chao, S.L. (2013). Decolorization of azo dye reactive black B by *Bacillus cereus* strain HJ-1. *Chemosphere*. 90(7): 2109-2114.

Mali, P.L.; Mahajan, M.M.; Patil, D.P.; Kulkarni, M.V. (2000). Biodecolourisation of members of triphenylmethane and azo group of dyes. *J. Sci. Ind. Res.,* 59: 221–224.

Moosvi, S.; Keharia, H.; Madamwar, D. (2005). Decolorization of textile dye reactive violet 5 by a newly isolated bacterial consortium RVM 11.1. *World J. Microbiol. Biotechnol.,* 21: 667–672.

Patil; P.S.; Shedbalkar, U.U.; Kalyani, D.C.; Jadhav, J.P. (2008). Biodegradation of Reactive Blue 59 by isolated bacterial consortium PMB11. *J. Ind. Microbiol. Biotechnol.,* 35: 1181–1190.

Sandhya, S.; Sarayu, K.; Uma, B.; Swaminathan, K. (2008). Decolorizing kinetics of a recombinant *Escherichia coli* SS125 strain harbouringazoreductase gene from *Bacillus latrosporus*RRK1. *Bioresour. Technol.,* 99(7): 2187–2191.

Tripathi, A.; Srivastava, S.K. (2011). Ecofriendly Treatment of Azo Dyes: Biodecolorization using Bacterial Strains. *Int. J. Biosci. Biochem. Bioinfo.,* 1(1): 37-40.

Verma, P.; Madamwar, D. (2003). Decolorization of synthetic dyes by a newly isolated strain of *Serratia marcescens*. *World J. Microbiol. Biotechnol.,* 19: 615–618.