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Evidence of Biodegradation of Reactive Red Textile Azo Dye in an Anoxic/Aerobic Bioremediation System

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ABSTRACT

The biodegradation of reactive red (RR) textile azo dye was investigated. Three bacterial strains, *Enterobacter cloacae*, *Pseudomonas* sp. and *Bacillus* sp. were used to decolourize and/or degrade the dye. A small bench-scale suspended bed bioreactor was used to study the capacity of these three bacterial strains to decolourize the dye solutions added as 5 successive supplements. The degradation of azo dyes was judged by the formation of aromatic amines (first product of dye biodegradation). All bacterial strains tested in this study produced aromatic amines under anoxic conditions. Additional evidence for biodegradation of RR textile azo dye was determined using 3 bioassay methods. Six test strains contributing to soil fertility were grown in spent media obtained from RR biodegradation. The growth of each test strain on biodegradation products was as high as the growth on the same specific media for each strain. This is evidence for the removal of toxicity in the biodegradation products. Another bioassay method used the germination of plant seeds to test dye phytotoxicity. The biodegradation of RR removed the phytotoxic effects of the dye on wheat (*Triticum aestivum* Giza '167') and berseem clover (*Trifolium alexandrinum* L.) seed germination. The chemical oxygen demand (COD), which indicates the organic load in the medium, decreased from 2048 to 599 ppm under aerobic conditions. The bacterial growth after biodegradation of RR increased indicating the breakdown of organics. A continued decrease in the COD value indicates a steady biodegradation of the anoxic biodegradation products as the growth conditions turned aerobic. This study shows that the biodegradation of a toxic textile azo dye, RR, is possible by using potent bacterial strains in a sequential anoxic/aerobic bioremediation system.

Keywords: bacteria, COD, phytotoxic, suspended bed bioreactor

INTRODUCTION

In recent years, the textile industry discharges about 50% of the textile azo dyes in a free state as factory effluent and eventually to the surrounding environment around the world (Pandey *et al.* 2007). Azo compounds constitute the largest and the most diverse group of synthetic dyes and are widely used in a number of industries such as textile, food, cosmetics and paper printing (Pandey *et al.* 2007). Effluents containing reactive azo dyes cause serious environment pollution (Darwesh *et al.* 2008). Therefore, such effluents must be treated before discharging into the environment to remove the dye toxicity from textile effluents (Wafaa *et al.* 2003; Umbuzeiro *et al.* 2005; Wafaa 2006; Corso and de Almeida 2009). However, the azo dyes are generally recalcitrant to biodegradation due to their xenobiotic nature (Darwesh *et al.* 2008). Microorganisms, being highly versatile, are expected to develop enzyme systems for the decolourization and mineralization of azo dyes under certain environmental conditions (Pandey *et al.* 2007). Anoxic degradation of various azo dyes (reactive black, sulfonated green, reactive blue and acid fast red) by mixed aerobic and facultative anaerobic microbial consortia have been reported (Khehra *et al.* 2005; Moosvi *et al.* 2005; Mohan *et al.* 2009). Regardless of the fact that many of these cultures are able to grow aerobically, degradation was achieved only under anaerobic conditions (Chen *et al.* 2003). A sequential anaerobic–aerobic process using a mixed culture of bacteria isolated from textile dye effluent-contaminated soil was used to degrade reactive and sulfonated azo dyes (Supaka *et al.* 2004). Exposing synthetic dye wastewaters to a combination of anaerobic and aerobic conditions showed that the majority of colours were removed in the anaerobic process, whereas the chemical oxygen demand (COD) was removed in the subsequent aerobic process (Supaka *et al.* 2004; Ali *et al.* 2009).

The biodegradation of azo dyes starts by the cleavage of the dye structure releasing aromatic amine (Khehra *et al.* 2006). Dyestuff toxicity (i.e. mortality, genotoxicity, mutagenicity and carcinogenicity) were studied on both aquatic organisms and mammals (Van der Zee 2002; Mahmoodi and Arami 2009). Chronic effects of dyestuffs, especially of azo dyes were seldom directly mutagenic or carcinogenic (Van der Zee 2002). Perey *et al.* (2002) and Pinheiro *et al.* (2004) reported that not all aromatic amines are toxic and carcinogenic. Moawad and Wafaa (2003) studied the phytotoxity of two textile dyes (direct brown and polar red) at 300 ppm to the water used to irrigate clover (*Trifolium alexandrinum* L.), wheat (*Triticum aestivum* 'Giza 167') and tomato (*Solanum lycopersicum*) grown in a pot experiment. The results indicate a decrease in plant growth and dry weight accumulation in the treatments receiving dye compared with control plants irrigated with Nile River water. Moawad *et al.* (2003) also studied the phytotoxicity of different soluble textile dyes by measuring the relative changes in seed germination of four plants, clover (*T. alexandrinum*), wheat (*T. aestivum* 'Giza 167'), tomato (*S. lycopersicum*) and lettuce (*Lactuca sativa*). Kalyani *et al.* (2008) reported that *Sorghum vulgare* and *Phaseolus mungo* seeds were more sensitive to the toxic effect of dye than the products obtained after dye decolourization. The aim of this study was to evidence the biodegradation of toxic textile azo dyes by using potent bacterial strains in a sequential anoxic/aerobic bioremediation system.

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Original Research Paper

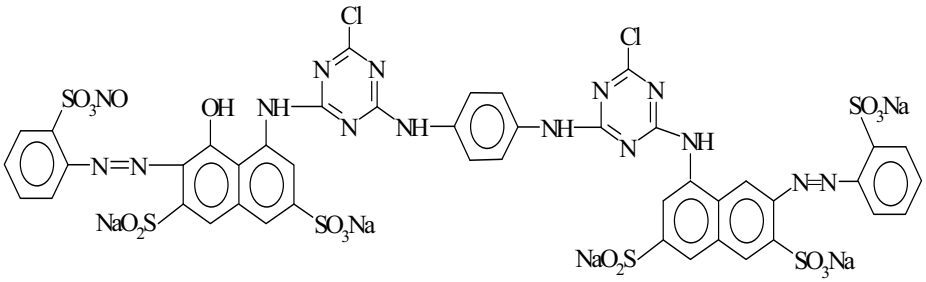


Fig. 1 Structure of Reactive Red azo dye.

MATERIALS AND METHODS

Microorganisms

Three bacterial isolates active in dye removal were isolated from wastewater- and soil-contaminated samples on modified mineral salt medium (MSM) (Darwesh *et al.* 2008). Several strains of test microorganisms (*Azotobacter* sp., *Pseudomonas* sp., *Rhizobium* sp., *Rhizobium leguminosarum* biovar *trifolii*, *Rhizobium legumenosarum* biovar *vicia* and *Cryptococcus albidus*) were obtained from the Agricultural Microbiology Department, National Research Center, Egypt.

Plant seeds

Wheat (*Triticum aestivum* 'Giza 167') and berseem clover (*Trifolium alexandrinum* L.) seeds were obtained from the Agronomy Department, Faculty of Agriculture, Cairo University.

Dye determination

The specific absorption spectrum of dye solution at λ = 555 nm was assayed using an LBK spectrophotometer model 4054. The structure of reactive red (RR) dye is shown in **Fig. 1**. RR has a molecular weight of 1437 and contains 36.8% C and 13.6% N.

Measurement of dye removal capacity

Dye decolourizing activity was expressed in terms of decolourization percentage and was determined by measuring the absorbance at 555 nm against the original colour of the medium. Decolourization activity (%) was calculated as follows:

D% = (Reading of [C] D – Reading of [S] D)/Reading of [C] D × 100

D = Decolourization, C = control and S = sample.

Continuous removal of successive dye additions in a laboratory-scale suspended bed bioreactor

Pseudomonas 131, *Enterobacter* 141 and *Bacillus* isolates were used in this study. A small-scale suspended bed bioreactor was used to study the dynamics of dye removal at the bench scale level. A glass column (4 cm in diameter × 70 cm long) was used as a model for the suspended bed bioreactor for biotreatment of RR. Bacteria were pre-cultured on modified MSM: 2.6 g/L glucose, 1 g/L ammonium sulfate, 0.1 g/L sodium chloride, 1 g/L di-potassium phosphate, 0.5 g/L mono-potassium phosphate, 0.2 g/L magnesium sulfate and 0.5 g/L yeast extract (Darwesh *et al.* 2008). After 3 days' incubation, the bacterial suspension was added to fill 10% of the total column volume. The remaining 90% of the column volume was filled with dye solution (300 ppm) prepared in water and amended with yeast extract (0.5 g/l). Several dye additions were added to the bioreactor, each of which was calculated to keep the dye concentration at 300 ppm in the bioreactor. The bioreactor was kept static at room temperature (27-30°C) for 20 days. New dye additions (100 ml each) were added after the total decolourization took place. A 100-ml of sample was withdrawn for measuring the colour removal before adding a similar dose of dye solution to the bioreactor. A total of five × 100-ml amendments were made. Biodegradation was determined by the diazodization test at the end of the experiment mentioned previously by Darwesh *et al.* (2008).

Microbial toxicity of dye biodegradation products

Six test strains mentioned in microorganisms section of the Materials and Methods were used in this experiment. Each was grown in spent media obtained from the bioremediation of RR in a suspended bed bioreactor receiving 5 sequential amendments of the dye. The direct effect of RR on the growth of each strain was used as a control. One loop of each strain was applied as inoculum in flasks containing 50 ml of MSM modified medium amended with RR dye (300 ppm) as the carbon and nitrogen source or RR bioremediation products. All flasks were incubated at 28 ± 2°C on a shaker at 150 rpm. Optical density was measured at 660 nm after 2, 3, 4, 5 and 6 days using a Jenway 6405 UV/visible spectrophotometer (UK).

Removal of RR dye phytotoxicity

The phytotoxic effects of RR dye at 300 ppm in water (control) and products of RR dye after bioremediation were tested on germination and early seedling growth of two plants (berseem clover and wheat). These crops are commonly cultivated in the Nile Delta region where textile industry plants are located. The seeds were germinated in sterile 10 cm Petri dishes, layered with sterile filter paper. Seeds were sterilized according to Moawad and Wafaa (2003) before transferring to the surface of the paper in the Petri dishes (10 seeds per dish). Seeds of clover and wheat were irrigated with 1 ml of dye solution (300 ppm) for each dish. One ml of the same solution was applied every day to the surface of the filter paper. Each treatment was replicated three times. Seeds germinated in water-irrigated Petri dishes were used as a control. All dishes were kept at room temperature for 9 days. Germination of seeds was recorded daily. Seeds were considered germinated when both epicotyl and hypocotyls emerged. The germination percentage was estimated after 3, 6 and 9 days. The shoot and root length of seedlings were measured on the same days. The results of the seed germination test were analyzed statistically. The effect of dye solutions on microbial strains was performed in media containing tap water (as control), RR solution (300 ppm) and/or the product of bacterial degradation.

Bacterial growth and COD changes under aerobic conditions in media containing anoxic dye bioremediation products

RR was subjected to anoxic biodegradation by *Pseudomonas* sp. strain 131. The products of biodegradation under anoxic conditions by this strain after 4 days of incubation in a suspended bed bioreactor at room temperature (24-26°C) were transferred (50 ml) into 150-ml flasks. The flasks were inoculated with 1 ml of 3-days-old culture of the three tested strains: *Pseudomonas* sp. strain 131, *Bacillus* sp. strain 147 and *Enterobacter cloacae* strain 141. The flasks were incubated in an incubator shaker (150 rpm) at 28°C and samples were collected after 7 and 15 days to test the changes in COD and optical density (OD). COD was measured using a Hach spectrophotometer test kit and OD using a Jenway 6405 UV/Visible- spectrophotometer (UK) at 660 nm.

Experimental design, sampling and statistical analyses

The data generated from germination test performed included measuring the shoot and root elongation was statistically analyzed using analysis of variance and LSD test "Mstate" Michigan State University.

RESULTS

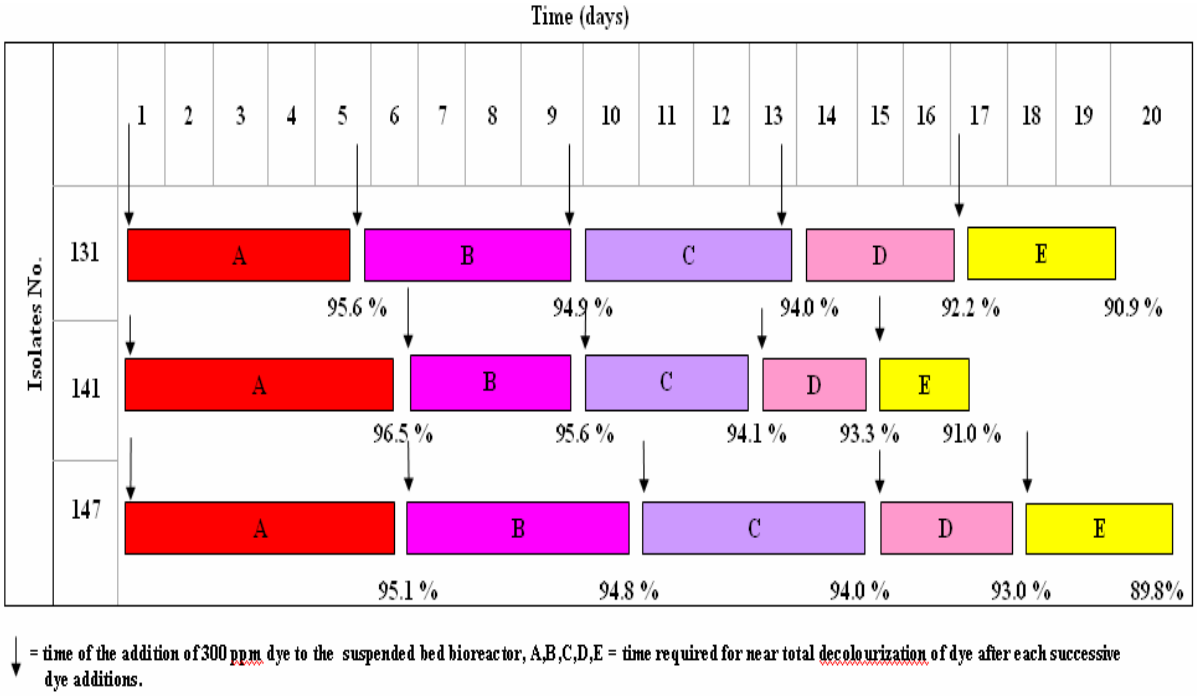
Continuous removal of successive dye additions to laboratory-scale suspended bed bioreactor

The aim of this part was to study a bioremediation system for the biotreatment of textile dyes residues in order to reduce the pollution of the agricultural environment by toxic dye residues reaching water and soil near industrial textile sites. Three bacterial isolates were identified as efficient biological agents capable of removing and/or biodegrading RR under specific conditions (Darwesh *et al.* 2008). The results indicate that an anoxic state is the key factor in the induction of enzymatic biodegradation of this recalcitrant organic pollutant. The isolates used were *Pseudomonas* sp. 131, *E. cloacae* 141 and *Bacillus* sp. 147. **Fig. 2** shows the decolourization and/or biodegradation of RR. The three bacterial strains were capable of decolourizing the dye solutions efficiently throughout 5 successive additions. *E. cloacae* strain 141 was the most efficient among the three strains since it showed accelerated dye removal starting from the second dye addition on day 6. All four other dye additions to the bioreactor containing this strain were decolourized faster than the other two strains. It is likely that this strain took the first 6 days to adapt to the dye amendment after which the enzymatic system of the strain operated more efficiently. The fifth addition of the dye to the bioreactor was 91% decolourized (the earliest) at 16 days from the start of the experiment. The other strains (*Pseudomonas* sp. 131 and *Bacillus* sp. 147) also efficiently biodegraded RR and bioremoved the colour. However, the 5th and last dye addition was decolourized by 91 and 90%, respectively on the 19th and 20th day of the start of the experiment. The data in **Fig. 2** shows that the time required for bioremoval/biodegradation of the same amount of added dye was markedly shortened with the repeated dye application which shows the potential application of this system in dye bioremediation technology. Successive applications of the dye did not stop dye removal until the 20th day of treatment, which indicates that the biodegradation products do not seem to be toxic to any of the three strains. This makes the three strains in general and *E. cloacae* strain 141 in particular ideal for use in designing and up-scaling a bioremediation system to treat industrial textile wastewater containing RR residues. It is evident that this type of work will require additional in-depth studies to find out the possible interactions with other ingredients in industrial wastewater. However, the value of these results is important if we take into consideration that these strains were isolated from textile industrial wastewaters loaded with mixtures of other pollutants and likely that these strains are adapted to other possible pollutants discharged from the same industry.

The evidence of RR dye biodegradation after successive addition of dyes was judged by the formation of aromatic amine (the first product of azo dye breakdown) in the suspended bed bioreactor media. The chromatogram showed the peak of aromatic amine measured at 275 nm (data not shown).

Microbial toxicity of dye biodegradation products

Six test strains belonging to different species namely *Azotobacter* sp., *Pseudomonas* sp., *Rhizobium* sp., *Rhizobium leguminosarum* biovar *trifolii*, *Rhizobium leguminosarum* biovar *vicia* and *Cryptococcus albidus* strains were used in this experiment. The main objective of this experiment was to test the effect of RR biodegradation products on the growth of several ecologically important tested microbes and whether the biodegradation products were toxic to these microbes or whether they could enrich the medium with nutritive elements released from the degraded organic pollutant to the tested microorganisms. Each strain was grown in spent media obtained from the bioremediation of RR in a suspended bed bioreactor receiving 5 sequential amendments of the dye, as shown in **Fig. 2**. As a control for this experiment the direct effect of the original RR dye solution on the growth of each strain was included. The results in **Fig. 3** show that the growth of microbial strains used as test organisms were limited throughout the experiment compared with the growth of these microbes on specific media for each tested strain. The growth of each strain on the biodegradation products increased with time to almost the same growth obtained on the specific media for each strain. This shows that certain nutritive components have been rel-



↓ = time of the addition of 300 ppm dye to the suspended bed bioreactor, A,B,C,D,E = time required for near total decolourization of dye after each successive dye additions.

Fig. 2 Decolourization/biodegradation of Reactive Red dye successive additions to the growth medium in suspended bed bioreactor.

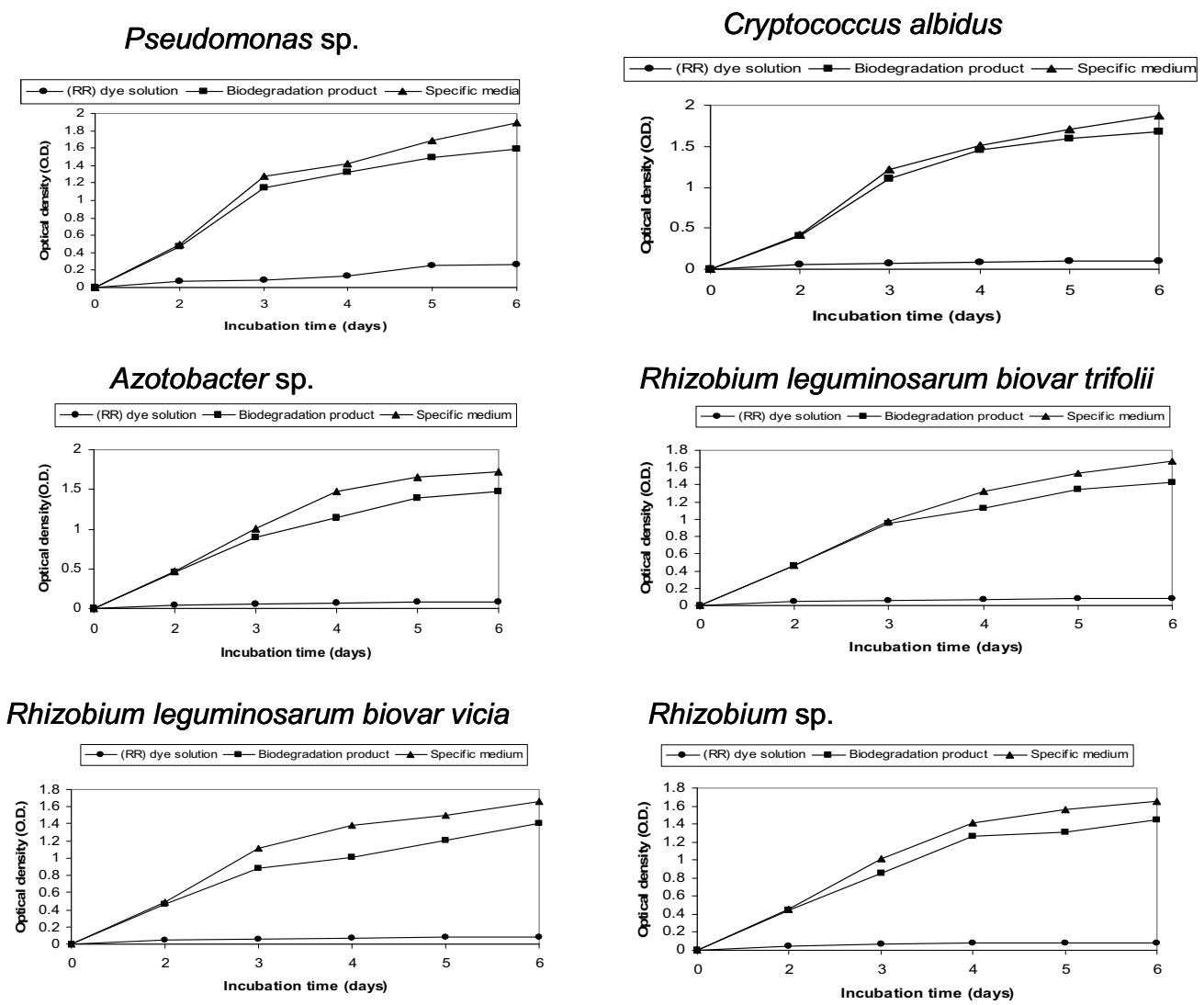


Fig. 3 Growth of six microbial strains on Reactive Red (RR) textile dye and its biodegradation products.

eased from the dyes to the media and supported the growth of the test strains indicating the partial biodegradation of the dye in the bioreactor. Similar trends were observed with the growth of all six strains on the bioremediation products of the partial degradation of RR in the bioremediation system. This shows that the bioremediation products are not toxic to any of the strains used in this bioassay.

Removal of RR phytotoxicity

The effect of RR bioremediation/biodegradation products on seed germination is presented in **Table 1**. The effect on wheat seed germination shows that the dye bioremediation products obtained after bacterial biodegradation did not have a toxic effect on the germination of seeds as well as the shoot and root elongation throughout the experiment. An exception is the early root elongation (3 and 6 days) where elongation was not comparable with the control. However, after 9 days the root length increased to the same value as that in the control.

The bacterial degradation of RR dye removed the dye toxicity and the germination of berseem clover seeds as well as shoot and root elongation throughout the experiment did not differ significantly from the control. The bioremediation/biodegradation products improved the germination, shoot and root elongation of berseem clover. This clearly show the real need to bioremediate the dye containing effluents before discharging it into the agricultural ecosystem to insure safe disposal of such pollutants And to avoid adverse effect of the highly toxic industrial organic pollutants.

Chemical Oxygen Demand (COD) changes associated with growth of bacteria under aerobic conditions in media containing anoxic dye bioremediation products

The obtained results show that primary degradation of RR textile azo dye under anoxic conditions in suspended bed bioreactor resulted in the formation of aromatic amines (**Fig. 4**) indicating initiation of biodegradation of azo dye in this growth phase. Further biodegradation of anoxic biodegradation products was studied by changing the conditions from anoxic to aerobic one. In this experiment the RR azo dye was first subjected to anoxic biodegradation by *Pseudomonas* sp. strain 131 which was the fastest, among other strains, in the early decolourization of RR dye in suspended bed bioreactor (**Fig. 4**). Within 4 days this strain was able to remove 95.6% of the dye colour (**Fig. 4**). Subjecting the anoxic biodegradation products to the three strains under aerobic condition the results obtained show that, the chemical oxygen demand has changed in the growth media from 2048 ppm initial values to 1600, 1609 and 1596 ppm after 7 days for isolates 147, 131and 141, respectively. This was accompanied by marked increase in optical density indicating active growth of all the three strains on the biodegradation products under aerobic conditions (**Table 2**). Incubation for 15 days resulted in further decrease in COD values being 690, 801 and 599 for the same strains. This indicates that, around 80% of the organic load in the anoxic biodegradation products was biodegraded by the tested strains under aerobic conditions.

Table 1 Effect of RR dye and products of its bioremediation on germination of berseem clover and wheat seeds as well as their root and shoot elongation.

Parameters	Days	Wheat			Berseem clover		
		RR	Bd	Control (water)	RR	Bd	Control (water)
Germination %	3	90	100	100	80	100	100
	6	90	100	100	80	100	100
	9	90	100	100	60	100	100
LSD at 0.05		7.17	2.62	5.24	6.41	6.92	6.92
Root elongation (cm)	3	2	5	8	1	2	4
	6	2.5	11	12	1	3.5	5
	9	1.75	16	16	0.75	5.0	6
LSD at 0.05		2. 069	2.62	2.62	1.308	1.603	2.267
Shoot elongation (cm)	3	1.2	2	2	2.6	2.6	3
	6	1.8	3.3	3.1	2.6	4.0	5
	9	2.1	5.3	5.2	2.6	6.0	7
LSD at 0.05		1.375	3.463	3.463	1.2	1.397	1.308

Table 2 Biodegradation of anoxic bioremediation products under aerobic conditions.

Strains	Chemical oxygen demand			Optical density		
	Initial [a]	7 days	15 days	Initial [b]	7 days	15 days
Bacillus sp. (147)	2048	1600	690	0.275	1.604	1.557
Enterobacter cloacae (141)	2048	1596	599	0.348	1.602	1.568
Pseudomonas sp. (131)	2048	1609	801	0.293	1.557	1.456

a = COD of anoxic biodegradation products.
b = Optical density of the initial bacterial growth.

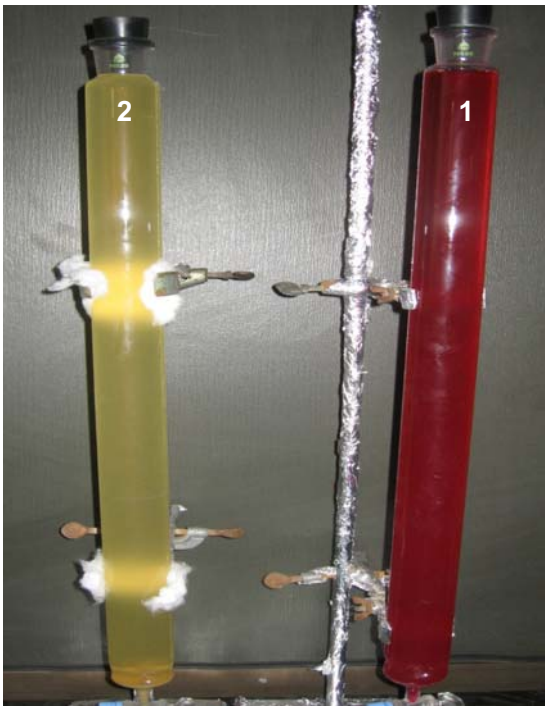


Fig. 4 Reactive Red dye biodegradation by *Pseudomonas* sp. isolate 131 under anoxic conditions in suspended bed bioreactor. 1 = control of RR dye, 2 = products of RR dye biodegradation after 4 days.

DISCUSSION

A small-scale suspended bioreactor was designed to test the biodegradation of the reactive red (RR) textile azo dye. Three bacterial isolates were identified as efficient biological agents capable to remove and/or biodegrade the RR dye under specific conditions (Darwesh *et al.* 2008). These isolates were identified as *Pseudomonas* sp. 131, *Enterobacter cloacae* 141 and *Bacillus* sp. 147. Three bacterial strains, namely *Pseudomonas* sp. 131, *Enterobacter cloacae* 141 and *Bacillus* sp. 147 were used in this study. The bench scale bioreactor was used as a step toward up-scaling the bioremediation process from lab bench scale to pre-pilot. Sequential five additions of dye were applied to the bioreactor. *Enterobacter cloacae* strain 141 was the best among other bacteria since it showed acceleration of dye removal starting from the second dye addition after six days. All other four dye additions to the bioreactor containing this strain were decolourized faster as compared with the other two bacteria. It is likely that this strain took the first 6 days for adaptation to the dye applied after which the enzymatic system of the strain operated more efficiently. It is documented that the microbial adaptation to the environment is necessary to enhance the biological process (Chen *et al.* 2003; Ali *et al.* 2009). Most of the studies on colour removal were carried out by using synthetic wastewater containing single textile dyestuff (Sen and Demirer 2003), however, textile wastewater contains different other chemicals. Regardless the criticism against use of synthetic wastewater that hardly represent the real conditions; the use of synthetic wastewater dye stuff is essential for better understanding the required bioremediation conditions for each specific dye. In a study by Sen and Demirer (2003) who investigated the anaerobic treatment of a cotton textile dye wastewater in a fluidized bed reactor (FBR) with pumice as the support material, they found that around 82, 94 and 59%, for COD, BOD and colour removal were possible respectively at 24 h hydraulic retention time (HRT). They suggested that this type of work will require additional in depth studies to find out the possible interactions with other ingredients in the industrial wastewater. However the value of these results is important if we take into consideration that these strains were isolated from textile industrial wastewaters which indicate that these strains are adapted to other possible pollutants discharged from textile industry with textile dye residues (Lin *et al.* 2010). The evidence of dye biodegradation after the successive addition of dyes was judged by the formation of aromatic amines in the suspended bed bioreactor media. Additional evidence of dye biodegradation was based on the growth and multiplication of several living organisms as bioindicators on dye biodegradation products. For this six strains belonging to different species namely *Azotobacter* sp., *Pseudomonas* sp., *Rhizobium* sp., *Rhizobium leguminosarum biovar trifolii*, *Rhizobium leguminosarum biovar vicia* and *Cryptococcus albidus* strains were used as test strains. Each strain was grown in spent media obtained from the bioremediation/biodegradation of RR dye in suspended bed bioreactor receiving 5 sequential amendments of the dye for up to19 days. Controls for this experiment were dye without treatment as negative control and the growth of each bacterial strain on specific media standard for each strain as positive one. The results showed that, the growth of microbial strains used as indicators was limited on dye containing media throughout

the experiment as compared with the growth on the specific media for each test strain. The growth of each strain on the obtained bioremediation/biodegradation product was almost similar to the bacterial growth on the specific media. This shows that, certain carbon and nitrogen nutritive components have been released from the dyes to the media and supported the growth of the test strains indicating that, certain biodegradation processes of the dye took place in the bioreactor under anoxic/aerobic conditions created with sequential static phase followed by steering/mixing phase with each additional dye amendments (Lin *et al.* 2010). Similar trends were observed with the growth of all six strains tests. All tested strains are microflora known to play a role in improving plant growth and soil fertility as they take part in the biotransformation of organic materials and nutrients in the soil. The nitrogen-fixing bacteria *Rhizobium* and *Azotobacter* can be manipulated ecologically, agronomically, edaphically and genetically to improve legume productivity and, as a consequence, soil fertility (John *et al.* 1995). *Pseudomonas* and diverse biodegradation of organic materials in soil which ultimately contribute to the improvement of soil fertility, also yeasts represent portion of soil microflora and thought to activate the root growth and plant production (Dhir *et al.* 2003; Singh and Ward 2004; Bronze 2006). Two plants were also used in bioassay test to judge the successful removal/biodegradation of RR azo dye. The use of dye bioremediation products showed that, the biodegradation of dye with bacteria removed the phytotoxic effect of the dye to the germination of wheat seed. The bacterial degradation of RR dye removed the dye toxicity and the germination of berseem clover seeds as well as the shoot and roots elongation throughout the experiment did not differ significantly from the control. In general, the bioremediation/biodegradation products improved the germination and shoot as well as root elongation of berseem clover. Similar results were obtained by Moawad and Wafaa (2003). Additional evidence of dye biodegradation is clear from the changes of COD values of dye solution in the bioreactor with time. The continued decrease in COD value indicates steady biodegradation of the anoxic biodegradation products under the aerobic conditions. The follow up of bacterial growth in the bioreactor shows that the constant OD values at 15 days of incubation as compared with those at 7 days indicate that, the growth of the strain has entered the stationary phase; however, the cells are still capable to biodegrade the organics exciting in the growth media. Sponza and Isik (2002) reported that, the COD removal efficiencies of 28, 42, and 90% were obtained at 11 days in the aerobic completely stirred tank reactors (CSTR). Optimum sludge retention time was 11 days in aerobic reactor. A 90–95% colour and 40–60% COD removal efficiencies were obtained depending on the applied organic loadings in the up flow anaerobic sludge blanket (UASB) reactor. The remaining COD was removed with a treatment efficiency of 85–90% in the aerobic CSTR reactor. Isik and Sponza (2006, 2008) reported that, in the sequential aerobic stage the significant part of total aromatic amines (TAA) was removed successfully while the colour removal slightly increased with TAA removal efficiencies of 70–85% at total hydraulic retention times (HRTs) of 8.85 and 6.05 days, respectively. Increases in HRT provide enough time for partial mineralization of COD and intermetabolites in anaerobic and/or anaerobic/aerobic systems. Treating synthetic dye wastewater with the combination of anaerobic and aerobic process showed that, the majority of colours are removed by this process, whereas the chemical oxygen demand (COD) is removed only in the subsequent aerobic process (Rajaguru *et al.* 2000; Supaka *et al.* 2004). The introduction of new bioremediation technologies is essential to safe environment from pollution. The textile industry is becoming more vital for the economy of almost all developing countries. The toxic effluents discharged from this industry have to be properly treated using appropriate bioremediation technologies before discharging it into the surrounding ecosystem to insure safe disposal of industrial wastes. This clearly shows the need for the development of cost effective bioremediation technologies for different industrial activities including textile industry.

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