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Potential Use of Probiotic Consortium Isolated from Kefir for Textile Azo Dye Decolorization

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Azo dyes are recalcitrant pollutants, which are toxic, carcinogenic, mutagenic and teratogenic, that constitute a significant burden to the environment. The decolorization and the mineralization efficiency of Remazol Brillant Orange 3R (RBO 3R) was studied using a probiotic consortium (*Lactobacillus acidophilus* and *Lactobacillus plantarum*). Biodegradation of RBO 3R (750 ppm) was investigated under shaking condition in Mineral Salt Medium (MSM) solution at pH 11.5 and temperature 25°C. The bio-decolorization process was further confirmed by FTIR and UV-Vis analysis. Under optimal conditions, the bacterial consortium was able to decolorize the dye completely (>99%) within 12 h. The color removal was 99.37% at 750 ppm. Muliplex PCR technique was used to detect the *Lactobacillus* genes. Using phytotoxicity, cytotoxicity, mutagenicity and biototoxicity endpoints, toxicological studies of RBO 3R before and after biodegradation were examined. A toxicity assay signaled that biodegradation led to detoxification of RBO 3R dye.

Keywords: Azo dye, RBO 3R, *Lactobacillus*, mutagenicity, biotoxicity, multiplex PCR

Introduction

Durable water supply is a rising demand in today's world due to water scarcity and pollutants discharged into the environment from industries. Among the pollutants released into the environment, dyes can be considered as one of the most dangerous contaminants. [1]. Dyes are toxic, carcinogenic, mutagenic, and teratogenic to humans and other life forms [2]. Synthetic dyes are divided into different classes on the basis of the chromophore group chemical structure [3]. Azo dyes are synthetic aromatic compounds having one or more azo bonds (N=N) [4]. Therefore, there is a rising demand for successful and cost-effective technologies to remove dyes from the global water supply. The textile industry is the most important user of synthetic dyes consuming about 56% of the total annual world production (7×10^5 tons) [5–7]. Azo dyes that account for about 50% of available produced industrial dyes, are extensively used in a variety of industries such as textiles, paper, plastics, cosmetics, and leather [8].

Biodegradation is an environmentally friendly and cost-competitive solution to chemical putrefaction processes [9]. Moreover, decolorization and degradation can also detoxify the effluent. Recent integral work has discovered the subsistence of an extensive variety of microorganisms capable of decolorizing a wide range of azo dyes such as *Aspergillus versicolor* [10], *Trametes versicolor* [11], *Pseudomonas putida* K1, *Serratia proteamaculans* SL14 [12] and *Phormidium* sp. [13].

The aim of the present study was to assess the performance

of a probiotic consortium (*Lactobacillus acidophilus* and *Lactobacillus plantarum*) to decolorize Remazol brillant orange 3R (RBO 3R). The *Lactobacilli* were characterized by multiplex PCR, and the degradation-produced metabolites were also evaluated using FTIR and UV-Vis spectroscopy. The metabolites were analyzed for their phytotoxicity, mutagenicity, biotoxicity and cytotoxicity.

Materials and Methods

Dye and Chemicals

Remazol brillant orange 3R and all chemicals were purchased from Sigma–Aldrich (USA), and were of the highest purity available. The chemical structure of RBO 3R was shown in Fig. 1.

Bacterial Strains and Culture Conditions

L. acidophilus and *L. plantarum* used in this study were isolated from Tibetan kefir. *Lactobacilli* were investigated for RBO 3R degradation using MRS medium in a cell-free system at pH 11.5 under shaking conditions. Conditioned medium was collected after 24 h and centrifuged at 300 ×*g* at 4°C for 10 min to eliminate bacteria. The supernatant was centrifuged at 1,200 ×*g* at 4°C for 20 min and 10,000 ×*g* at 4°C for 30 min. The EV pellet was collected by ultracentrifugation at 100,000 ×*g* at 4°C for 70 min and suspended in PBS [14].

Adaptation

The adaptation was carried out by exposing *L. acidophilus* and *L. plantarum* gradually to the highest concentrations of RBO 3R [6]. The *Lactobacilli* were cultivated at 30°C for 24 h in 500 ml Erlenmeyer flasks containing g/l peptone (10); malt extract (8); yeast extract (4); sodium acetate (0.2); magnesium sulfate (0.05); potassium phosphate (2) Tween 80 (1 ml) and glucose (20) (pH 6.0). During the present study, MRS *Lactobacillus* broth concentration was reduced from 90% (w/v) to 0% (w/v) and finally the organisms were provided with RBO 3R solution as a unique source of carbohydrate. The adaptation experiments were performed at (T:25°C, pH 11.5 and shaking condition).

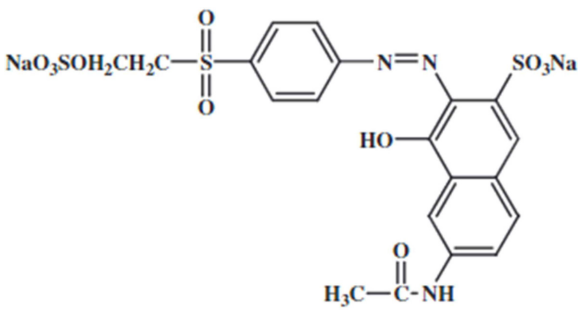

Fig. 1. Chemical structure of Remazol Brillant Orange 3R (λ_{max} (nm) = 494).

Preparation of DNA Templates for PCR Assays and Multiplex PCR Assay

Extraction of genomic DNA assay was achieved according to Kwon *et al*. [15]. Multiplex PCR detection of specific sequences of *lactobacillus* species was performed as previously described [15]. Amplification program began firstly with a 2 min initial cycle at 94°C. Secondly, the denaturation step was executed at 94°C through 35 cycles of 20 s. Thirdly, the annealing step was achieved at 51°C for 40 s; and the extension step was carried out at 68°C for 30 s. Finally, the elongation step was completed at 68°C for 7 min in a DNA thermal cycler (GenAmp PCR System 9700-Applied Biosystems Int., USA) [15–17].

Biodegradation of Remazol Brillant Orange 3R and Color Removal

Decolorization of RBO 3R by *L. acidophilus* and *L. plantarum* was studied at different concentrations (750, 800, 850, 900, 950, and 1,000 ppm) at pH 11.5, T 25°C and aerobic condition. Dye removal was determined according to Ayed *et al*. [18–23].

Phytoxicity, Mutagenicity, Cytotoxicity, and Biotoxicity Assessment Tests

Phytotoxicity test was carried out in order to evaluate the toxicity of RBO 3R and its decolorization metabolites. Tests were carried out, as described previously by Ayed *et al*. [18] on seeds of Tunisian *Saragolla*. The RBO 3R and metabolic product extracted by ethyl acetate were dissolved separately in distilled water to obtain a solution of 1,000 ppm. The mutagenicity assay with *Salmonella Typhimurium* TA98 and TA100 was performed as described by Ayed *et al*. [2]. The cytotoxicity test was carried out using human epithelial cells (Hep G2) and dedication of cell viability according to Ayed *et al.* [23–25]. The biotoxicity tests were performed with *Artemia salina* according to Ayed *et al*. [6]. The RBO 3R was analyzed in a concentration series (100%, 75%, 50%, and 25%). After 24 h incubation, the number of dead larvae was counted.

UV–Vis and FTIR Spectral Analyses

To determine the decolorization efficiency, the samples were taken using syringe at different time intervals and centrifuged at 13,000 ×*g* for 5 min. A Hitachi UV/visible spectrophotometer (Hach DR 2000) was used for spectral analysis of RBO 3R and its decolorization products after incubation for 12 h, and the spectrum was recorded from wavelength of 200–800 nm.

Fourier-transform infrared spectroscopy (FTIR) was applied to dictate the functional groups participating in the degradation of RBO 3R. The metabolites were characterized by FTIR (Perkin-Elmer, Spectrum One) according to Ayed *et al*. [7].

Results and Discussion

Isolation, Identification and Adaptation of Microorganisms

Bacterial strains having dye decolorization capacity were secluded from kefir using sequential dilution and spread

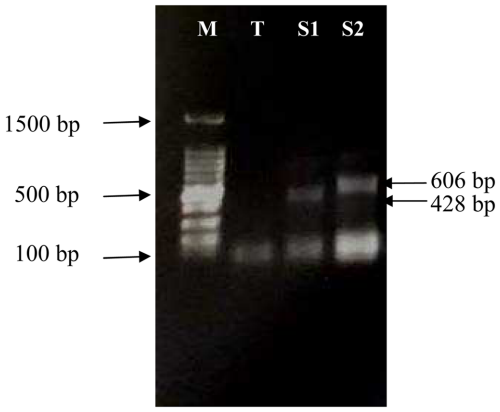

Fig. 2. Agarose gel electrophoresis (0.7%) of PCR products from multiplex PCR assays.

Multiplex PCR assays were performed with a mixture of seven species-specific or group-specific primers for *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. gasseri*, *L. plantarum*, *L. reuteri*, and *L. rhamnosus*). Lane T: negative control; lane M: 100 bp-DNA ladder DNA molecular size marker. S1: *Lactobacillus plantarum*; (amplified PCR product: 428 bp); S2: *Lactobacillus acidophilus* (amplified PCR product: 606 bp).

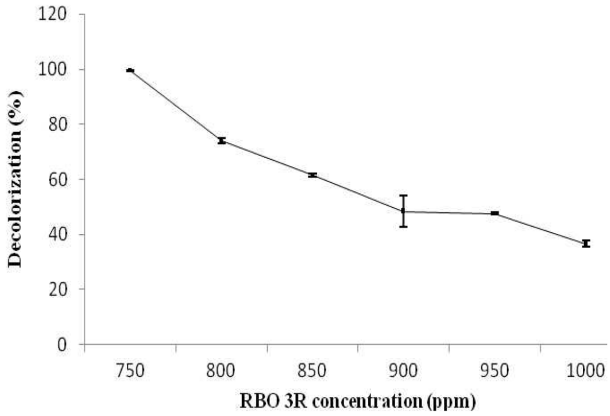

Fig. 3. Effect of RBO 3R initial concentration on decolorization by *Lactobacillus acidophilus* and *Lactobacillus plantarum* (12 h).

Temperature = 25°C, pH = 11.5, and [bacterium]o = 5×10^7 cells/ml.

plate method based on their ability to create a clear zone in the nutrient agar plate containing RBO 3R dye. The constituted primer set was implemented to identify two *Lactobacillus* strains. Fig. 2 showed the amplified PCR products of *L. plantarum* giving a 428 bp band and *L. acidophilus* giving a 606 bp band. In addition, the primer design, through the integration of the rRNA sequences, will help to design other identification systems. The identified species S1 (*L. plantarum*) and S2 (*L. acidophilus*) were adapted by raising the concentration of dye and at the same time reducing the concentration of nutrients. During the initial phase, the *Lactobacilli* were provided with suitable energy source and the growth was observed to be good.

Effect of the Concentration of RBO 3R on the Decolorization of Remazol Brillant Orange 3R

The effect of initial RBO 3R concentration on the decolorization was investigated, with *L. acidophilus* and *L. plantarum*, and it was noticed that the percentage of decolorization was reduced, thus raising the initial concentration of RBO 3R from 750 to 1,000 ppm (Fig. 3). It was noticed that after 12 h incubation, only 36.56% decolorization was observed when using the initial dye concentration of 1,000 ppm. It was well observed that RBO 3R completely decolorized (99.37%) and biodegraded during the shaking condition regardless of its initial concentration of 750 ppm. These results are in contrast with Vaigan *et al*. [26] when adjusting the initial concentration of reactive blue (Brill Blue KN-R) from 20 to 40 ppm, in anaerobic condition, the removal efficiencies of reactive dye decreased from 57% to 31%.

Bacterial degradation of azo dye has been explored as a financially viable option. Many azo dye decolorizing bacteria have been reported earlier, *e.g*., *Citrobacter* sp. [27], *Aeromonas* sp. [28], *Comamonas* sp. [29], *Pseudomonas* sp. [30], *Enterobacter* sp. [31] and *Alishewanella* sp. [32].

UV-VIS and FTIR Spectral Analysis

Fig. 4 displayed a typical time dependent UV-VIS spectrum of RBO 3R solution during decolorization dye solution treatment with *L. acidophilus* and *L. plantarum*. The absorbance peaks corresponded to the dye biodegradation. Their abatement indicated that the dye has been eliminated. The RBO 3R spectrum in visible region exhibited a major peak with a maximum absorbance at 494 nm (Fig. 4). According to Ayed *et al*. [5], the biodecolorization of dyes can be engendered by adsorption to the bacteria or the biodegradation. As shown in Fig. 4 the major absorbance peak approximately disappeared within 12 h, indicating the degradation of aromatic and the naphthalene rings [33]. The results indicated the proficiency of these *Lactobacilli* towards the degradation of recalcitrant dye molecules.

The comparison of FTIR spectra before and after decolorization (Fig. 5) displayed substantial peak difference signifiance to various operability. The bands at 1,635, 1,535, 1,141, 1,049, 950, and 750 cm^{-1} disappeared after 12 h of biodegradation treatment. These bands corresponded to-OH, -N = N-, C-N, characteristic of a sulfonic group (R-SO3) stretching vibration and N-H bond vibrations respectively [34] present in the initial molecule, and their

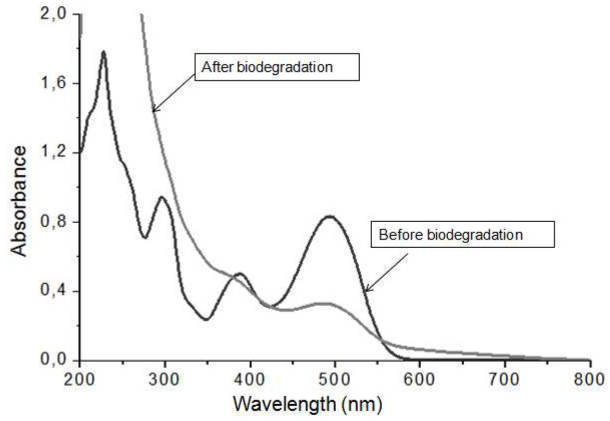

Fig. 4. UV–vis spectra of Remazol brillant orange 3R (λ_{max} (nm) = 494) biodegraded by *Lactobacillus acidophilus* and *Lactobacillus plantarum* bacteria (12 h).
Temperature = 25°C, pH = 11.5, [RBO 3R]o = 750 ppm and [bacterium]o = 5×10^7 cells/ml.

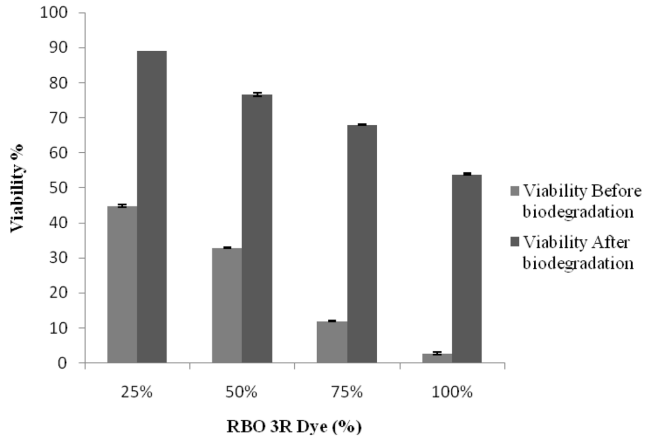

Fig. 6. Cytotoxicity of different concentrations (25–100%) of Remazol Brillant Orange 3R before and after biodegradation by MTT assay using Hep G2 cell line.

disappearance indicated that oxidative treatment broke down the molecules. The disappearance of peaks and appearance of some new peaks in the FTIR spectra of the sample study after biodegradation gives evidence of azo bond segmentation and generation of different metabolites [35, 36]. After the biodegradation, a new band increased to 1,125 and 575 cm^{-1}. This indicated the presence of secondary aromatic amines [37], that originated after dye molecule biotransformation. Other peaks in the spectrum after biodegradation, suggested the production of oximes and imines [38].

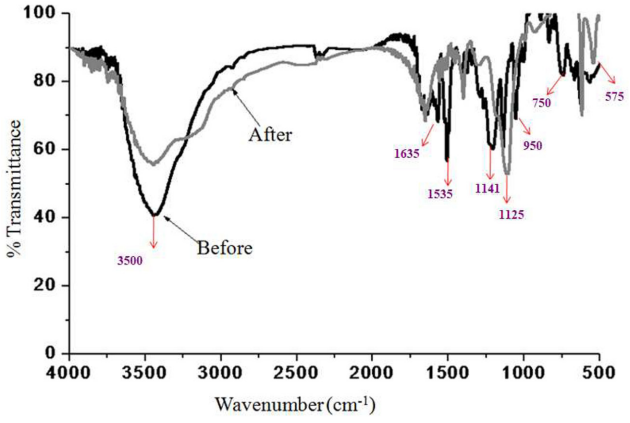

Fig. 5. FTIR spectra of Remazol brillant orange 3R before and after biodegradation (12 h) by *Lactobacillus acidophilus* and *Lactobacillus plantarum* bacteria (12 h).
Temperature = 25°C, pH = 11.5, [RBO 3R]o = 750 ppm, and [bacterium]o = 5×10^7 cells/ml.

Phytoxicity Assay Using Tunisian *Soragolla*

Previous studies reported that several azo dyes and their metabolites, such as sulfonated and unsulfonated aromatic amines, were a main source of environmental pollutants [39, 40]. The relative sensitivity towards the dye and metabolites in rapport to Tunisian *Saragolla* was explored (Table 1). The germination suggested a little toxicity of the degradation products to plants. There was also a difference between length of root and shoot of *Soragolla* treated by RBO 3R and its degradation products. Ayed *et al*. [5, 7, 18, 19] described that germination of *Triticum aestivum* was of less phytoxicity with azo and triphenylmethane dyes compared to metabolic product extracted. These results

Table 1. Effect of different concentrations of RBO 3R before (BT) and after (AT) biodegradation on seed germination, root length, shoot length of early seedling of Tunisian *Saragolla*.

RBO 3 R	Germination	Root length (cm)	Shoot length (cm)
Control	100 ± 0	3.4 ± 0.2	5.4 ± 0.1
BT 25%	55 ± 0.4	1.4 ± 0.1	1.5 ± 0.1
BT 50%	45 ± 0.3	1.0 ± 0.1	1.3 ± 0.2
BT75%	40 ± 0.3	0.5 ± 0.01	1.0 ± 0.1
BT 100%	20 ± 0.2	0.2 ± 0.01	0.7 ± 0.01
AT 25%	100 ± 0.1	3.4 ± 0.2	5.3 ± 0.4
AT 50%	100 ± 0.1	3.3 ± 0.1	5.0 ± 0.5
AT 75%	100 ± 0.1	3.0 ± 0.1	4.8 ± 0.6
AT 100%	95 ± 0.2	3.0 ± 0.1	4.5 ± 0.5

Values are mean ± SD (n = 3).

indicated that the decolorization metabolites were less phytotoxic compared with the original dye.

Cytotoxicity Assay Using Human Epithelial Cell Line (Hep G2)

The cytotoxicity of RBO 3R dye solutions (before and after biodegradation) was also defined using human epithelial (Hep G2) cell line by MTT assays. The percentages for the survival ability of human cell line by MTT assay were 45%, 33%, 12%, and 3% at 1/4, 1/2, 3/4 and 1/1, respectively, after 12 h exposure to dye solution before biodegradation. However, after biodegradation the metabolic product solution showed a reduction in cell survivability and the noted percentages were 89%, 77%, 68%, and 54% at the same concentrations (Fig. 6). Cell survivability assay by MTT showed that the cytotoxicity of RBO 3R dye solutions increased after being treated with *L. acidophilus* and *L. plantarum*. The decrease of MTT evaluates the functional intactness of mitochondria based on the enzymatic reduction of tetrazolium salt by the mitochondrial dehydrogenase of viable cells [25]. These results indicated that the decolorization products were less cytotoxic compared with the original dye.

Biotoxicity Test Using *Artemia salina*

From the results for *A. salina* in Fig. 7, showing the toxicity of RBO 3R before and after biodegradation, it can be observed that the non-treated dye showed a mortality rate of 79.33%, 68.66%, 56.66%, and 45.66% at 1/4, 1/2, 3/4, and 1/1 respectively after 12 h of contact with the dye solution before biodegradation. However, after biodegradation at shaking condition, dye solutions showed a reduction in mortality of *A. salina* and the recorded percentages were 33.33%, 24.66%, 14.33%, and 10.66% at the same concentrations (Fig. 7). Consequently aerobic treatment of azo dyes was deemed to be more efficient and secure than anaerobic treatment. Previous research showed the competence of *Lactobacillus* strains to decay azo dyes either in static [41] or in micro-static condition [42]. *L. acidophilus* and *L. plantarum* were found to be able to aerobically decolorize azo dye with reduced toxicity, indicating that the degraded intermediates are detoxified during the treatment. Similar results were also reported for *Exiguobacterium* sp. RD3 [43], *Sphingomonas paucimobilis* [5], and *L. paracase* CL1107 strains [44].

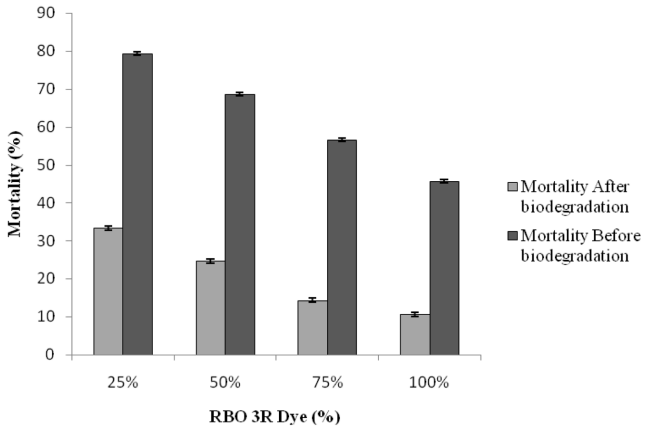

Fig. 7. Biotoxicity of different concentrations (25–100%) of RBO 3R before (BT) and after (AT) biodegradation using *Artemia salina*.

Mutagenicity Assessment

In the experiments, prior to mutageni, the inductor bacteria do not effect its viability study, it was confirmed that the different stands of extracts and added molecules. After biodegradation of the dye the mutagenicity decreased when compared to the untreated pure dye even with the S9 metabolizing system at the highest tested dose of 200 mg/assay. However, no mutagenicity of the dye products was observed after shaken incubation with *Lactobacillus* in the presence of S9 metabolizing system (Table 2). The absence of mutagenic effect of azo dye metabolite samples from aerobic cultures with probiotic could be clarified by the presence of an adequate level of dissolved oxygen that might limit the azo-reductase action and raise the oxygenase activity; thus granting the decolorization of the dye aromatic amines to which the mutagenic effects may be assigned. Marques *et al*. [45], Nakayama *et al*. [46], Sweeney *et al*. [47], Erdoğdular and Dilek [48] and Jayalakshmi and Jeyanthi [49] reported that carcinogenicity via mutagenicity of some azo dyes and allied aromatic amines could be engendered

Table 2. Mutagenic activity of Remazol Brillant Orande 3 R, before and after biodegradation evaluated by the Ames and assay using *Salmonella typhimurium* TA98 in the presence of S9 mix.

Revertants number		
Tested compound	TA98	RM for TA98
Dye	Before biodegradation	
RBO 3R[1]	17.4 ± 2.12	1.03
RBO 3R[2]	18 ± 1.41	1.07
	After biodegradation	
RBO 3R[1]	47 ± 7.48	2.62
RBO 3R[2]	58 ± 5.14	3.25
SR	17.5 ± 3.5	-
Positive control	17.5 ± 3.5	1

Positive control (PC), TA98+S9 = 100 µg/plate, SR: spontaneous revertant

by the production of vacant groups.

In conclusion, the present study displayed the ability of *L. acidophilus* and *L. plantarum* to decolorize Remazol Brillant Orange 3R. The formulated probiotic *Lactobacilli* indicated a stronger color removal yield as compared to pure cultures, which revealed a complementary interaction among various isolated *lactobacilli*. The consortium performed a significantly stronger reduction in color (99.37%) at 750 ppm, pH 11.5, T 25°C and shaking condition in little time (12 h). Phytotoxicity, cytotoxicity, biotoxicity and mutageinicity indicated the efficiency of the *Lactobacilli* in decreasing the toxicity of the azo dye. Nevertheless, the toxicity study displayed that RBO 3R was degraded into non-toxic compounds by the probiotic consortium.

Acknowledgments

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Jayalakshmi R, Jeyanthi J. 2019. Simultaneous removal of binary dye from textile effluent using cobalt ferrite-alginate nano composite: performance and mechanism. *Microchem. J.* **145:** 791-800.
2. Ayed L, Bakir K, Ben Mansour H, Hammami S, Cheref A, Bakhrouf A. 2017. In vitro mutagenicity, NMR metabolite characterization of azo and triphenylmethanes dyes by adherents bacteria and the role of the "cna" adhesion gene in activated sludge. *Microb. Pathog*. **103:** 29-39.
3. Mahmoud MS, Mostafa MK, Mohamed SA, Sobhy NA, Nasr M. 2017. Bioremediation of red azo dye from aqueous solutions by *Aspergillus niger* strain isolated from textile wastewater. *J. Environ. Chem. Eng*. **5:** 547-554.
4. Thung WE, Ong SA, Ho LN, Wong YS, Ridwan F, Lehl HK, *et al.* 2018. Biodegradation of acid orange 7 in a combined anaerobic-aerobic up-flow membrane-less microbial fuel cell: mechanism of biodegradation and electron transfer. *Chem. Eng. J*. **336:** 397-405.
5. Ayed L, Khelifi E, Ben Jannet H, Miladi H, Cheref A, Achour S, *et al*. 2010c. Response surface methodology for decolorization of azo dye Methyl Orange by bacterial consortium: produced enzymes and metabolites characterization. *Chem. Eng. J*. **165:** 200-208.
6. Ayed L, Achour S, Bakhrouf A. 2011a. Application of the Mixture Design to decolorize effluent textile wastewater using continuous stirred bed reactor. *Water SA* **37:** 21-26.
7. Ayed L, Mahdhi A, Cheref A, Bakhrouf A. 2011b. Decolorization and degradation of azo dye Methyl red by *Sphingomonas paucimobilis*. *Desalination* **274:** 272-277.
8. Saratale RG, Saratale GD, Chang JS, Govindwar SP. 2011. Bacterial decolorization and degradation of azo dyes: a review. *J. Taiwan Inst. Chem. Eng*. **42:** 138-157.
9. Verma P, Madamwar D. 2003. Decolorization of synthetic dyes by a newly isolated strain of Serratia marcescens. *World J. Microbiol. Biotechnol*. **19**: 615-618.
10. Tastan BE, Ertugrul S, Donmez G. 2010. Effective bioremoval of reactive dye and heavy metals by *Aspergillus versicolor*. *Bioresour. Technol*. **101:** 870-876.
11. Aksu Z, Kılıç NK, Ertugrul S, Donmez G. 2007. Inhibitory effects of chromium (VI) and Remazol Black B on chromium (VI) and dyestuff removals by *Trametes versicolor*. *Enzyme Microb. Technol*. **40:** 1167-1174.
12. Mahmood R, Sharif F, Ali S, Hayyat MU. 2014. Enhancing the decolorizing and. degradation ability of bacterial consortium isolated from textile effluent affected area and its application on seed germination. *Sci. World J*. **2015:** 628195.
13. Sadettin S, Donmez G. 2007. Simultaneous bioaccumulation of reactive dye and chromium (VI) by using thermophil *Phormidium* sp. *Enzyme Microb. Techcnol*. **41:** 175-180.
14. Karri RR, Tanzifi M, Yaraki MT, Sahu JN. 2018. Optimization and modeling of methyl orange adsorption onto polyaniline T nano-adsorbent through response surface methodology and differential evolution embedded neural network. *J. Environ. Manag*. **223:** 517-529.
15. Kwon S, Yang EH, Yeon SW, Kang BH, Kim TY. 2004. Rapid identification of probiotic *Lactobacillus* species by multiplex PCR using species-specific primers based on the region extending from 16S rRNA through 23S rRNA Hyuk. *FEMS Microbiol. Lett*. **239:** 267-275.
16. Jin C, Luo P, Zuo H, Chen J, Chen M, Wang W. 2012. *Vibrio zhanjiangensis* sp. nov. isolated from sea water of shrimp farming pond, Antonie Van Leeuwenhoek **101:** 743-751.
17. Seo MK, Park EJ, Ko SY, Choi EW, Kim S. 2018. Therapeutic effects of kefir grain *Lactobacillus*-derived extracellular vesicles in mice with 2,4,6-trinitrobenzene sulfonic acid-induced inflammatory bowel disease. *J. Dairy Sci*. **101:** 8662-8671.
18. Ayed L, Chaieb K, Cheref A, Bakhrouf A. 2009. Biodegradation of triphenylmethane dye Malachite Green by *Sphingomonas paucimobilis*. *World J. Microbiol. Biotechnol*. **25:** 705-711.
19. Ayed L, Cheriaa J, Laadhari N, Cheref A, Bakhrouf A. 2009. Biodegradation of Crystal Violet by an isolated *Bacillus* sp. *Annals. Microbiol*. **59:** 267-272.

20. Ayed L, Achour S, Khelifi E, Cheref A, Bakhrouf A. 2010. Use of active consortia of constructed ternary bacterial cultures via mixture design for Congo Red decolorization enhancement. *Chem. Eng. J*. **162:** 495-502.
21. Ayed L, Chaieb K, Cheref A, Bakhrouf A. 2010. Biodegradation of triphenylmethane dyes by *Staphylococcus epidermidis*. *Desalination* **260:** 137-146.
22. Ayed L, Harbi B, Cheref A, Bakhrouf A, Achour S. 2010. Application of the Mixture Design to decolorize azo-dye Methyl Red to Design the Formulation of active consortia of bacterial cultures. *Water Sci. Technol*. **62:** 2837-2845.
23. Ayed L, Bekir K., Achour S, Cheref A, Bakhrouf A. 2016. Exploring bioaugmentation strategies for azo dye CI Reactive Violet 5 decolourization using bacterial mixture: dye response surface methodology. *Water. Environ. J*. http://dx.doi.org/10.1111/2016.wej.12216.
24. Maron DM, Ames BN. 1983. Revised methods for the Salmonella mutagenicity test. *Mutat. Res*. **113:** 173-215.
25. Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **65:** 55-63.
26. Vaigan AA, Moghaddam MRA, Hashemi HS. 2009. Effect of dye concentration on sequencing batch reactor performance. *J. Environ. Health Sci. Eng*. **6:** 11-16.
27. An SY, Min SK, Cha IH, Choi YL, Cho YS, Kim CH. 2002. Decolorization of triphenylmethane and azo dyes by *Citrobacter* sp. *Biotechnol. Lett*. **24:** 1037-1040.
28. Ren SZ, Guo J, Zeng GQ, Sun GP. 2006. Decolorization of triphenylmethane, azo, and anthraquinone dyes by a newly isolated *Aeromonas hydrophila* strain. *Appl. Microbiol. Biotechnol*. **72:** 1316-1321.
29. Jadhav UU, Dawkar VV, Ghodake GS, Govindwar SP. 2008. Biodegradation of Direct Red 5B, a textile dye by newly isolated *Comamonas* sp. UVS. *J. Hazard Mater*. **158:** 507-516.
30. Kalyani DC, Telke AA, Dhanve RS, Jadhav JP. 2009. Ecofriendly biodegradation and detoxification of Reactive Red 2 textile dye by newly isolated *Pseudomonas* sp. SUK1. *J. Hazard Mater*. **163:** 735-742.
31. Wang H, Zheng XW, Su JQ, Tian Y, Xiong XJ, Zheng TL. 2009. Biological decolorization of the reactive dyes Reactive Black 5 by a novel isolated bacterial strain *Enterobacter* sp. EC3. *J. Hazard. Mater*. **171:** 654-659.
32. Kolekar YM, Kodam KM. 2012. Decolorization of textile dyes by *Alishewanella* sp. KMK6. *Appl. Microbiol. Biotechnol*. **95:** 521-529.
33. Zhang J, Zhou Q, Ou L. 2011. Kinetic, isotherm, and thermodynamic studies of the adsorption of methyl orange from aqueous solution by chitosan/alumina composite. *J. Chem. Eng. Data* **57:** 412-419.
34. Wang S, Yang B, Liu Y. 2017. Synthesis of a hierarchical SnS2 nanostructure for efficient adsorption of Rhodamine B dye. *J. Colloid Interface Sci*. **507:** 225-233.
35. Phugare SS, Kalyani DC, Patil AV, Jadhav JP. 2011. Textile dye degradation by bacterial consortium and subsequent toxicological analysis of dye and dye metabolites using cytotoxicity, genotoxicity and oxidative stress studies. *J. Hazard. Mater*. **186:** 713-723.
36. Lade HS, Waghmode TR, Kadam AA, Govindwar SP. 2012. Enhanced biodegradation and detoxification of disperse azo dye Rubine GFL and textile industry effluent by defined fungal-bacterial consortium. *Int. Biodeterior. Biodegrad*. **72:** 94-107.
37. Ayed L, Ksibi I, Cheref A, Bakhrouf A. 2012. Response surface methodology for optimization of the treatment of textile wastewater by a novel bacterial consortium: enzymes and metabolites characterization. *Afr. J. Biotechnol*. **11:** 12339-12355.
38. Banerjee P, Barman SR, Sikdar D, Roy U, Mukhopadhyay A, Das P. 2017. Enhanced degradation of ternary dye effluent by developed bacterial consortium with RSM optimization, ANN modeling, and toxicity evaluation. *Desalination Water Treat*. **72:** 249-265.
39. Raj A, Kumar S, Haq I, Singh SK. 2014. Bioremediation and toxicity reduction in pulp and paper mill effluent by newly isolated ligninolytic *Paenibacillus sp. Ecol. Eng*. **71:** 355-362.
40. Kumari V, Kumar S, Haq I, Yadav A, Singh VK, Ali Z, *et al*. 2014. Effect of tannery effluent toxicity on seed germination a-amylase activity and early seedling growth of mung bean (*Vigna radiata*) seeds. *Int. J. Latest Res. Sci. Technol*. **3:** 165-170.
41. Chen BY, Lin KW, Wang YM, Yen CY. 2009. Revealing interactive toxicity ofaromatic amines to azo dye decolorizer Aeromonas hydrophila. *J. Hazard. Mater*. **166:** 187-194.
42. Seesuriyachan P, Takenaka S, Kuntiya A, Klayraung S, Murakami S, Aoki K. 2007. Metabolism of azo dyes by *Lactobacillus casei* TISTR 1500 and effects of various factors on decolorization. *Water Res*. **41:** 985-992.
43. Dhanve RS, Shedbalkar UU, Jadhav JP. 2008. Biodegradation of diazo reactive dye Navy Blue HE2R (Reactive Blue 172) by an isolated *Exiguobacterium* sp. RD3. *Biotechnol. Bioprocess. Eng*. **13:** 53-60.
44. Huang G, Wang W, Liu G. 2015. Simultaneous chromate reduction and azo dye decolourization by *Lactobacillus paracase* CL1107 isolated from deep sea sediment. *J. Environ. Manage.* **157:** 297-302.
45. Marques RCP, Medeiros SRB, Dias CS, Barbosafilho JM, Agnez-Lima LF. 2003. Evaluation of the mutagenic potential of yangambin and of the hydroalcoholic extract of *Ocotea duckei* by the Ames test. *Mutat. Res*. **536:** 117-120.
46. Nakayama T, Kimura T, Kodama MC. 1983. Generation of hydrogen peroxide and superoxide anions from active metabolites of naphthalamines and aminoazo dyes: its possible role in carcinogenesis. *Carcinogenesis* **4:** 765-769.
47. Sweeney EA, Chipman JK, Forsythe SJ. 1994. Evidence for direct-acting oxidative genotoxicity by reduction products of azo dyes. *Environ. Health Perspect*. **102:** 119-122.
48. Erdoğdular AO, Dilek KA. 2019. Bioremoval of reactive dye Remazol Navy by kefr grains. *Appl. Biol Chem*. **62:** 22.
49. Haq I, Raj A. 2018. Markandeya, Biodegradation of Azure-B dye by *Serratia liquefaciens* and its validation by phytotoxicity, genotoxicity and cytotoxicity studies. *Chemosphere* **196:** 58-68.