International Journal of Textile Science & Engineering

Neifar M, et al. J Textile Sci Eng : TSE-107.

DOI: 10.29011/TSE-107. 100007

Research Article

Modeling and Optimization of Textile Dye Decolourization Using Laccase-Producing, Alkali-Halotolerant Bacteria

Mohamed Neifar*, **Habib Chouchane, Amani Jdidi, Fatma Naili, Rania Ouertani, Mouna Mahjoubi, Atef Jaouani, Ameur Cherif**

LR Biotechnology and Bio-Geo Resources Valorization (LR11ES31), Higher Institute for Biotechnology - University of Manouba Biotechpole of Sidi Thabet, Tunisia

***Corresponding author:** Mohamed Neifar, LR Biotechnology and Bio-Geo Resources Valorization (LR11ES31), Higher Institute for Biotechnology - University of Manouba Biotechpole of Sidi Thabet, 2020, Sidi Thabet, Ariana, Tunisia. Tel: +21670527882; +21671537040; Fax: +21670527882; +21671537044; Email: mohamed.naifar@gmail.com

Citation: Neifar M, Chouchane H, Jdidi A, Naili F, Ouertani R, et al. (2018) Modeling and Optimization of Textile Dye Decolourization Using Laccase-Producing, Alkali-Halotolerant Bacteria. J Textile Sci Eng : TSE-107. DOI: 10.29011/TSE-107. 100007

Received Date: 02 January, 2018; **Accepted Date:** 10 January, 2018; **Published Date:** 19 January, 2018

Abstract

Microbial-assisted removal of hazardous textile dyes has been considered as an alternative and eco-friendly method compared to those of physico-chemical techniques. The present study was designed in order to isolate new alkali-halotolerant bacteria from Tunisian desert and to select efficient strains for degradation of textile azo dyes, Bezactiv Blue S-Matrix 150 and Tubantin Brown GGL. More than 50 halotolerant bacterial strains were isolated from desertic sediments of Ksar Ghilane oasis of which 16 showed a laccase activity. Laccase-producing bacteria exhibited different decolourization profiles towards the tested textile dyes. Phylogenetic analysis indicated that the best laccase-producing and dye-degrading strain was affiliated with the genus *Pseudomonas*. So, Response Surface Methodology (RSM) and three-level Central Composite Design (CCD) with four variables, namely dye concentration, pH, NaCl concentration and incubation time, were applied in order to optimize the decolourization process using *Pseudomonas resinovorans* G2 strain. The developed mathematical CCD model showed the effect of each factor and their interactions on Bezactiv Blue colour removal. The maximum decolourization yield was determined using NemrodW software and the predicted values were experimentally validated.

Keywords: Bioremediation; Extremophilic Bacteria; Laccase Enzyme; Response Surface Methodology; Textile Dyes

Introduction

Textile Wastewaters (TWWs) are considered as one of the major sources of pollution due to the great demand for textile products resulting proportional increase in production and application of synthetic dyes. Azo dyes represent the most common group of chemical dyes constituting 60-70% of more than 10,000 dyes used in textile industries [1]. It is estimated that about 2% and 10–15% of azo dyes are lost during manufacture and dyeing processes, respectively [2]. Release of theses dyes into the environment causes an adverse impact on different ecosystems because that they are considered to be toxic to aquatic biota and are reported to be carcinogenic to humans [1]. Although their contribution to organic load can sometimes be insignificant, even the presence of low levels of synthetic dyes would impart an intense colour to the TWWs [1,3]. Therefore, effective treatment of dye-containing wastewaters before discharge into the environment should be emphasized.

Compared with physical and chemical methods, biological treatment processes are generally considered as better alternatives for treatment of azo dye-containing wastewaters due to their lower cost, higher efficiency and less secondary pollution [4]. Generally, the TWW generated in reactive dyeing process not only marked by high colour, and pH but also carry a high load of salts. Although the majority of microorganisms were sensitive to harsh industrial conditions, some bacterial extremotolerant species still had shown the ability to degrade synthetic dyes or raw TWWs under alkaline and saline conditions [1,5,6]. The isolation and selection of highly efficient extremophilic bacteria are among the most important factors for effective biological treatment of TWWs [7-10]. *Response Surface Methodology* (RSM) *was proven* to be a powerful tool to optimize biodecolourization processes [5,11-14].

In this study, an alkali-halo tolerant bacterial strain capable of producing laccase and decolourizing various textile azo dyes was isolated, identified and characterized. Bezactiv blue S-Matrix 150 was chosen as the model azodye for further investigating the effects of different parameters on dye decolourization by means of RSM.

Materials and Methods

Dyes, Media and Chemicals

Bezactiv Blue S-Matrix 150 and Tubantin brown GGL used in this study were supplied from a textile factory in Nabeul, Tunisia and was of commercial quality. Stock dye solution was prepared at concentration of 1000 mg/L (w/v) sterilized by filter and added to the media in all manipulations. λ max of the dyes were determined in diluted dye aqueous solutions by using a scanning UV-vis spectrophotometer. Tryptic Soy Broth (TSB) (Sigma, 22092) and Tryptic Soy Agar (TSA) (Sigma, 22091) were used in the experiments. All chemicals used were of the highest purity available and of analytical grade.

Sampling, Isolation and Screening of Laccase Producing Extremophilic Bacteria

A composite soil sample from Ksar Ghilane oasis (N 32°59′012″, E 09°38′072″), an arid system in Tunisian desert, was prepared aseptically from five subsamples (1-10 cm deep) and collected from the arms and center of an X (each arm was 1 m in length). One cm soil from the ground surface was firstly removed to avoid contamination during sampling procedure. Samples were then transported to the laboratory in a cool box and stored at 4°C prior to processing [15].

Enrichment technique was applied for the isolation of moderately haloalkaliphilic bacteria as reported by El-Hidri et al. [16]. Plate assay method was performed to confirm the presence of laccase [5]**.** Enzyme detection was performed using TSA medium amended with 0.2% of 2,6 dimethoxyphenol (DMP) as enzyme substrate. The presence of brick colour around the colonies was considered as DMP oxidizing laccase secreting organism. Dye decolourization activities was revealed on TSA and TSB containing 100 mg/L of the dyes [17].

Enzyme Analysis

The laccase activity was measured by monitoring the oxidation of 5 mM DMP buffered with 50 mM phosphate (pH 8.0) at 469 nm for 1 min. To calculate enzyme activity an absorption coefficient of 27,500 $M^{-1}cm^{-1}$ was used. One unit of laccase activity was defined as the amount of enzyme required to oxidize 1 μM of 2,6-DMP oxidized per minute [5].

Molecular Identification and Phylogenetic Analysis of Selected Bacterium

The 16S rRNA gene from pure cultures was amplified using the following universal primers: S-D-Bact-0008-a-S-20/S-D-Bact-1495-a-A-20 according to the procedure described previously by Cherif et al. [18]. The 16S rRNA gene sequencing has been carried with an automated capillary ABI Biosystem 3130. Obtained sequences were initially compared to those available in GenBank database using BLAST (http://www.ncbi.nlm.nih.gov). Phylogenetic dendrograms were constructed by the neighbor-joining method and trees topology was evaluated by performing bootstrap analysis of 1000 data sets using MEGA 6.06 (Molecular Evolutionary Genetics Analysis) [5].

Decolourization Experiments and Optimization Study

1 ml of 1.0 % v/v (O.D. 600 nm ≈ 0.9) inoculum from the mother liquid culture was used to inoculate 50 ml of TSB medium contained dye and then flasks were incubated at 37°C on a rotatory shaker (150 rpm). The culture was centrifuged at 10000 rpm to separate the bacterial cell mass. The decolourization was quantified by measuring the decrease in absorbance of the dye using UV-Vis Spectrophotometer. Dyes of Bezactiv Blue S-Matrix 150 and Tubantin brown GGL had λmax values of 605 and 430 nm, respectively and then dye decolourization (%) was calculated.

A CCD consisting of 30 experiments was chosen for the optimization of Bezactiv Blue decolourization. Four independent variables, namely dye concentration (X_1), pH (X_2), NaCl (X_3) and incubation time (X_4), were evaluated at three levels (Table 1), and the percentage of Bezactiv Blue decolourization was the dependent variable (response). The following equation was used to establish the quadratic model:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_4 X_4 + b_{11} X_1^2 + b_{22} X_2^2 + b_{33} X_3^2 + b_{44} X_4^2 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{23} X_2 X_3 + b_{14} X_1 X_4 + b_{24} X_2 X_4 + b_{34} X_3 X_4$$

where Y is the response (% decolourization); Xi and Xj are uncoded independent variables; and b_0, b_j, b_{jj} and b_{jk} are intercept, linear, quadratic and interaction constant coefficients, respectively.

The FTIR analysis was done on Perkin Elmer, spectrum one instrument in the mid IR region of 400– 4,000 cm^{-1} with a scan speed of 16 (Spectrum One, Perkin Elmer, USA) as described by Si et al. [19].

Statistical Analysis

The generation and the data treatment of the CCD were performed using NemrodW software [20].

Results and Discussion

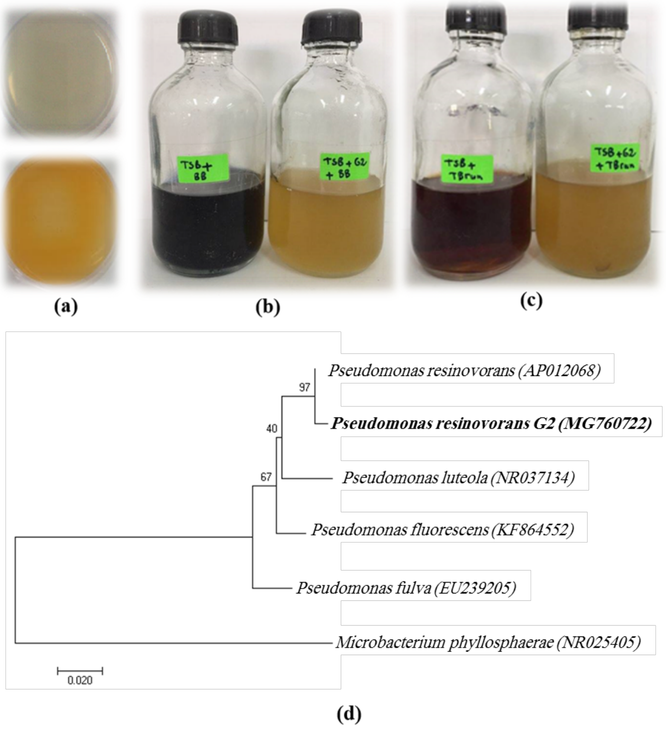
In the present investigation, 53 alkali-halotolerant bacteria (3% NaCl, pH 8), isolated from Ksar Ghilane oasis were screened for laccase activity using the chromogenic screening method on DMP supplemented TSA medium [5]. Of the 53 isolates, 16 formed brown zone around and above the colony which is a positive confirmation for their laccase activity secretion. Laccase producing isolates were subjected to secondary screening to investigate their ability to decolourize two synthetic dyes namely, Bezactiv Blue S-

Matrix 150 and Tubantin brown GGL. The isolates G7, G9, G12, G18, G19, G21, G22, G25, G27, G36, G45, G48, G49 were not able to decolourize both dyes (Table 1). In contrast, the highly laccase-producing isolate G2 was able to completely decolourize both tested dyes. Bezactiv Blue S-Matrix 150 was decolourized more rapidly than Tubantin brown GGL. Quantitative experiments in shake flask culture showed a laccase activity of 0.662±0.029 U/mL with complete Bezactiv Blue decolourization on 48h. The decolourization ability of laccase producing G2 against the two textile dyes are shown in (Figures1a-c).

The *16S* rRNA gene of the isolate G2, was amplified, sequenced and submitted to GenBank. The obtained sequence with accession number MG760722 was compared with those in the National Center for Biotechnology Information Nucleotide Sequence database by using the BLAST algorithm. Based on the phylogenetic analysis G2 strain was identified as *Pseudomonas resinovorans* via partial sequencing of 16s rRNA Figure 1d. Other pseudomonas strains were also reported for their ability to decolourize textile dyes in particular, *Pseudomonas aeruginosa* [7,21,22], *Pseudomonas fluorescens) [23] and Pseudomonas extremorientalis [5].*

Isolate code	DMP oxidation	Bezactiv blue oxidation	Tubantin brown oxidation
G7	++	+	+
G9	++	+	-
G12	+++	+	-
G18	+	+	-
G19	++	+++	-
G21	+	+	-
G22	+++	++	-
G23	+++	++	++
G25	+++	+++	-
G27	+++	++	-
G32	++	++	++
G36	+++	+++	-
G45	++	++	-
G48	+++	++	-
G49	+++	+++	-
G2	+++	+++	+++
(Oxidation scale: + 0-1 cm; ++ 1-3 cm; +++ 3-5cm; - absent)			

Table 1: Screening for laccase-producing and textile dye-degrading bacteria isolated from a desertic region in southern Tunisia.



Figures 1(a-d): (a) Detection of laccase activity of G2 strain grown on TSA supplemented with 0.2% DMP, 3% NaCl at pH8 for 3 days incubation at 37°C; (b) Bezactiv Blue decolourization and (c) Tubantin brown decolourization by G strain grown on TSB supplemented with 100 mg/L of the dye, during 2 days of incubation at 37°C. Phylogenetic analysis of 16S rRNA gene sequence of laccase-producing dye decolourizing isolate *Pseudomonas resinovorans* G2 based on 16S rDNA partial sequences. Phylogenetic dendrogram was evaluated by performing bootstrap analysis of 1000 data sets using MEGA 6.06 software. 16S rRNA sequence accession numbers of the reference strains are indicated in parentheses.

A CCD was chosen to determine the optimum requirement of enzyme (X_1), dye (X_2), salt (X_3) and time (X_4) for maximum dye decolourization by selected strain G2 (Table 2).

No. exp.	X_1	X_2	X_3	X_4	**Dye (mg/L)**	**pH**	**NaCl (%)**	**Incubation time (day)**	**Dye decolourization (%)**	
									Observed	**Predicted**
1	-1.0	-1.0	-1.0	-1.0	50.0	7.0	0.0	1.0	93.50	93.993
2	1.0	-1.0	-1.0	-1.0	250.0	7.0	0.0	1.0	77.10	79.570
3	-1.0	1.0	-1.0	-1.0	50.0	10.0	0.0	1.0	82.30	82.803
4	1.0	1.0	-1.0	-1.0	250.0	10.0	0.0	1.0	97.50	94.654
5	-1.0	-1.0	1.0	-1.0	50.0	7.0	5.0	1.0	70.20	70.347
6	1.0	-1.0	1.0	-1.0	250.0	7.0	5.0	1.0	2.20	-0.651
7	-1.0	1.0	1.0	-1.0	50.0	10.0	5.0	1.0	71.60	68.182
8	1.0	1.0	1.0	-1.0	250.0	10.0	5.0	1.0	21.00	23.458
9	-1.0	-1.0	-1.0	1.0	50.0	7.0	0.0	3.0	72.30	70.114
10	1.0	-1.0	-1.0	1.0	250.0	7.0	0.0	3.0	59.40	62.615
11	-1.0	1.0	-1.0	1.0	50.0	10.0	0.0	3.0	40.80	43.449
12	1.0	1.0	-1.0	1.0	250.0	10.0	0.0	3.0	62.10	62.225
13	-1.0	-1.0	1.0	1.0	50.0	7.0	5.0	3.0	69.20	71.843
14	1.0	-1.0	1.0	1.0	250.0	7.0	5.0	3,0	8.00	7.770
15	-1.0	1.0	1.0	1.0	50.0	10.0	5.0	3.0	56.40	54.203
16	1.0	1.0	1.0	1.0	250.0	10.0	5.0	3.0	17.10	16.404
17	-1.0	0.0	0.0	0.0	50.0	8.5	2.5	2.0	33.20	34.566
18	1.0	0.0	0.0	0.0	250.0	8.5	2.5	2.0	10.10	8.455
19	0.0	-1.0	0.0	0.0	150.0	7.0	2.5	2.0	67.20	63.499
20	0.0	1.0	0.0	0.0	150.0	10.0	2.5	2.0	58.80	62.221
21	0.0	0.0	-1.0	0.0	150.0	8.5	0.0	2.0	85.50	81.077
22	0.0	0.0	1.0	0.0	150.0	8.5	5.0	2.0	42.20	46.343
23	0.0	0.0	0.0	-1.0	150.0	8.5	2.5	1.0	53.50	56.543
24	0.0	0.0	0.0	1.0	150.0	8.5	2.5	3.0	44.40	41.077
25	0.0	0.0	0.0	0.0	150.0	8.5	2.5	2.0	42.90	46.860
26	0.0	0.0	0.0	0.0	150.0	8.5	2.5	2.0	45.20	46.860
27	0.0	0.0	0.0	0.0	150.0	8.5	2.5	2.0	48.70	46.860
28	0.0	0.0	0.0	0.0	150.0	8.5	2.5	2.0	49.80	46.860

Table 2: Experimental decolourization conditions of the CCD in coded and natural variables and the corresponding observed and predicted responses.

The mathematical expression of the relationship to the decolourization of Bezactiv blue S - Matrix 150 (Y) with the variables X_1, X_2, X_3 and X_4 is as follows:

$$Y = 46.86 - 13.05 X_1 - 17.37 X_3 - 7.73 X_4 - 25.35 X_1^2 + 16.00 X_2^2 + 16.85 X_3^2 + 6.57 X_1X_2 - 14.14 X_1 X_3 - 3.87 X_2X_4 + 6.34 X_3 X_4$$

Where Y are the G2 decolourization response (%); Xj: system variables (correspond to the different factors influencing the decolourization of bezactiv blue) and b_0, b_j, b_{jk} and b_{jj}: significant model coefficients.

ANOVA of the regression model demonstrated high significance of the model and insignificant lack of fit value (Table 3). The linear factors of X_1, X_3 and X_4; quadratic factor of X_1, X_2 and X_3 and interaction terms X_{12}, X_{13}, X_{24}, and X_{34} were found to be significant indicating that the model terms are limiting factors for BEZACTIV blue S-Matrix 150 decolourization (Table 4). The regression equation obtained indicated the *R-Squared, Adjusted R-Squared and Predicted R-Squared* values of 0.989, 0.977 and 0.941 respectively, suggesting an adequate adjustment of the quadratic model to the experimental data and indicating that the model could explain 98.9% of the variability in the response.

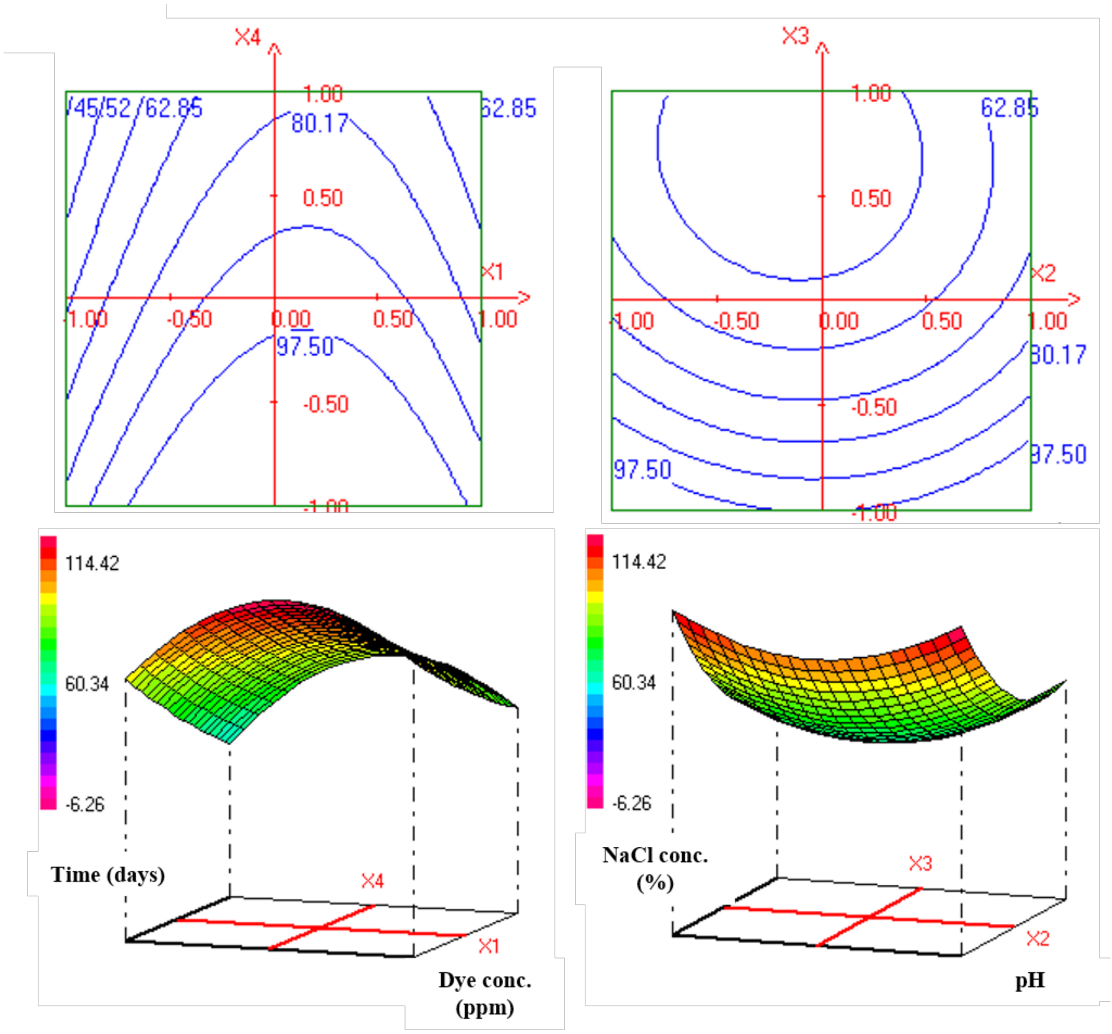
Name	Coefficient	F. Inflation	Stand. Dev.	t.exp.	Signification %
b0	46.860		1.331	35.21	***
b1	-13.056	1.00	0.907	-14.39	***
b2	-0.639	1.00	0.907	-0.70	50.0%
b3	-17.367	1.00	0.907	-19.14	***
b4	-7.733	1.00	0.907	-8.53	***
b11	-25.350	2.49	2.396	-10.58	***
b22	16.000	2.49	2.396	6.68	***
b33	16.850	2.49	2.396	7.03	***
b44	1.950	2.49	2.396	0.81	43.5%
b12	6.569	1.00	0.962	6.83	***
b13	-14.144	1.00	0.962	-14.70	***
b23	2.256	1.00	0.962	2.35	*
b14	1.731	1.00	0.962	1.80	9.2%
b24	-3.869	1.00	0.962	-4.02	**
b34	6.344	1.00	0.962	6.59	***
(***): significant at the level 99.9%; (**): significant at the level 99%; (*): significant at the level 95%.					

Table 3: Estimated effect, regression coefficient, and corresponding t and P values for Bezactiv Blue S-Matrix 150 decolourization by G2 strain in central composite design experiments.

Response	Source of variation	Sum of squares	Degrees of freedom	Mean square	Ratio	Significance
Y: Dye decolourization (%)	Regression	17252.9	14	1232.35	83.2019	***
	Residues	192.550	13	14.81		
	Validity	162.260	10	16.23	1.6071	38.1%
	Error	30.2900	3	10.09		
	Total	17445.4	27			
***: significant at the level of 99.9 %.						

Table 4: ANOVA for the response surface quadratic model.

The contour plots and response surface curves for the predicted response Y (Bezactiv blue decolourization yield), based on the second-order model were shown in Figure 2. They provided useful information about interactions between dye concentration, pH, salinity and incubation time and allowed an easy interpretation of the CCD results and prediction of the optimal levels *of* each variable for maximum Bezactiv blue decolourization. Indeed, Bezactiv blue decolourization enhanced by increasing dye concentration up to middle level (150 mg/L) and decreasing the NaCl concentration and incubation time. The decreasing dye decolourization at higher levels was probably as a result of possible enzyme inactivation at such high dye concentrations [5]. Decolourization of Bezactiv blue dye by G2 seems to be active at alkaline pHs (8-10). This result is advantageous to biologically treat TWWs because one of the most important characteristic of *TWWs* is their *alkalinity [24]*.

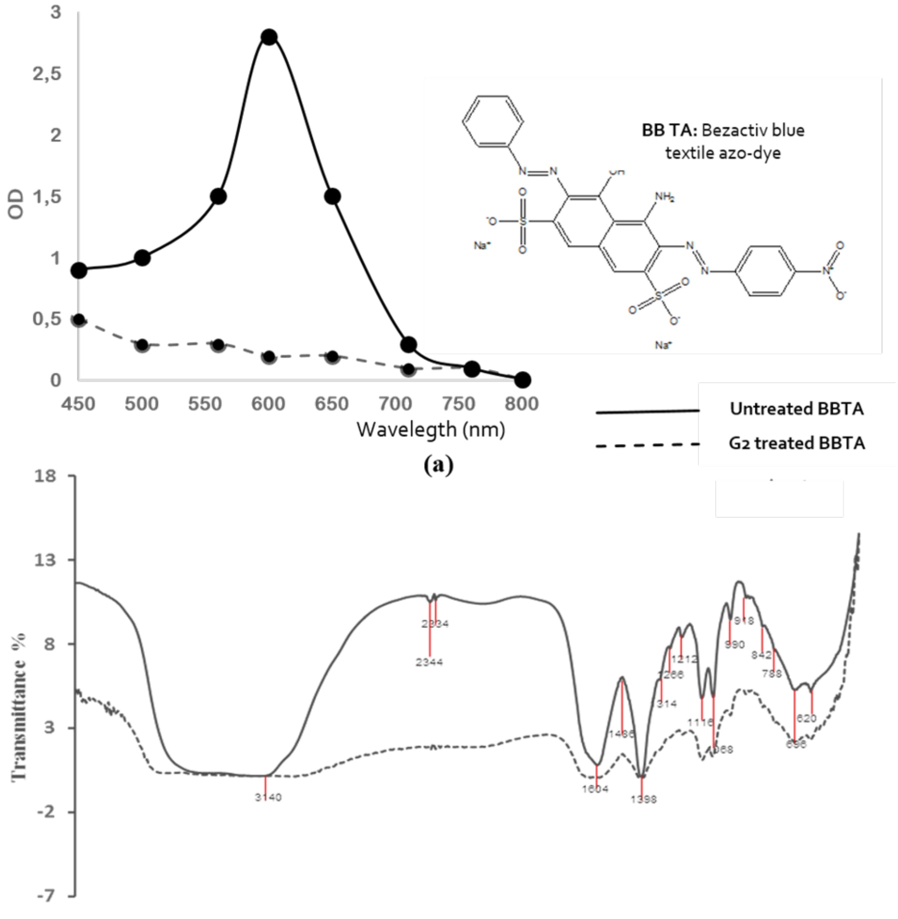


(a) **(b)**

Figure 2: Contour plots and response surfaces curves showing interactive effect of dye concentration and incubation time (a) as well as pH and NaCl concentration (b) on the decolourization of Bezactiv Blue S-Matrix 150 by G2 strain.

The optimum decolourization conditions of BEZACTIV blue S-Matrix 150, carried out numerically by using NemrodW software, are dye concentration 150 mg/L, pH 10, salt concentration 1% and incubation time 24h. The expected value of BEZACTIV blue decolourization yield was $y_{op} = 97.5\% \pm 2.5$. Additional experiments were carried out under the selected optimal decolourization conditions. It led to BEZACTIV blue decolourization yield equal to 98.2% ± 2.7, which was in close agreement with the predicted response value.

A noticeable difference was observed between the UV-vis and FTIR spectra of BEZACTIV blue S-Matrix 150 before and after G2 treatment (Figures 3a,3b). Comparison of FTIR spectrum of control dye with extracted metabolites from *Pseudomonas* strain after complete decolourization clearly indicated the degradation of dye BEZACTIV blue S-Matrix 150 by G2. The decolourization data obtained in the present study, support the observations of Singh et al. [25] and Mishra et al. [26] who isolated laccase-producing bacteria for decolourization of textile dyes.



(a)

(b)

Figures 3(a-b): The variation in UV-Vis **(a)** and FTIR **(b)** spectra of Bezactiv Blue S-Matrix 150 before and after decolourization by G2 strain.

Conclusions

The present study highlights the exploitation of laccase producing extremotolerant bacteria as an alternative method for treatments of dye containing effluents. The potential of selected bacterium *Pseudomonas resinovorans* G2 in the decolourization of textile dye BEZACTIV blue S-Matrix 150 was studied and optimized using response surface methodology. Dye removal efficiency was dependent on various physicochemical parameters such as dye concentration, initial pH, ionic strength and incubation time. The degradation of the dye was proved by FTIR spectra. Treatment of model BEZACTIV blue effluent using laccase producing alkali-Halotolerant *Pseudomonas resinovorans* G2 could be used as environmentally friendly and cost-effective bioprocess for TWWs management. Analytical and toxicological experiments are in progress in order to evaluate the degradation mechanism and the safety of the end-products. Application of extremotolerant bacteria as single strains and/*or as consortia* for treatment of real textile effluents needs further investigation.

Acknowledgements

The authors acknowledge financial support from the European Union in the ambit of the project MADFORWATER (H2020, GA 688320) and the Tunisian Ministry of Higher Education and Scientific Research in the ambit of the laboratory project LR11ES31.

References

1. Tan L, He M, Song L, Fu X, Shi S (2016) Aerobic decolorization, degradation and detoxification of azo dyes by a newly isolated salt-tolerant yeast *Scheffersomyces spartinae* TLHS-SF1. Bioresource Technology 203: 287-294.

2. Pearce CI, Lloyd JR, Guthrie JT (2003) The removal of color from textile wastewater using whole bacterial cells: a review. Dye Pigments 58: 179-196.

3. Singh KD, Sharma S, Dwivedi A, Pandey P, Thakur RL, et al. (2007) Microbial decolorization and bioremediation of melanoidin containing molasses spent wash. Journal of Environmental Biology 28: 675-677.

4. dos Santos AB, Cervantes FJ, Van Lier JB (2007) Review paper on current technologies for decolourisation of textile wastewaters: perspectives for anaerobic biotechnology. Bioresource Technology 98: 2369-2385.

5. Neifar M, Chouchane H, Mahjoubi M, Jaouani A, Cherif A (2016) *Pseudomonas extremorientalis* BU118: a new salt-tolerant laccase-secreting bacterium with biotechnological potential in textile azo dye decolourization. 3 Biotech 6:107.

6. Neifar M, Chouchane H, Masmoudi AS, Jaouani A, Cherif A (2017) Biotechnological potential of extremophilic laccases. In: Harris A (ed.). Laccase: Applications, Investigations and Insights. Nova Science Pub Inc, New York. Pg No: 7x10.

7. Albert K, Rose C, Nachiyar V (2011) Studies on Decolorisation of Textile Azo Dye, Mordant Black 17 Using *Pseudomonas aeruginosa* SBU7, Isolated From Textile Effluent. Green Technology and Environmental Conservation : 244-247.

8. Amoozegar MA, Mehrshad M, Akhoondi H (2015) Application of Extremophilic Microorganisms in Decolorization and Biodegradation of Textile Wastewater. In: Singh S (ed.). Microbial Degradation of Synthetic Dyes in Wastewaters. Environmental Science & Engineering. Springer, Cham.

9. Neifar M, Maktouf S, Ghorbel RE, Jaouani A, Cherif A (2015a) Extremophiles as source of novel bioactive compounds with industrial potential. In: Gupta V K, Tuohy M G, O'Donovan A, Lohani M (ed.). Biotechnology of Bioactive Compounds: Sources and Applications. Wiley, Hoboken, Pg No: 245–268.

10. Neifar M, Chouchane H, Jaouani A, Masmoudi AS, Cherif A (2015b) Extremozymes as efficient green biocatalysts in bioremediation of industrial wastewaters. In: Barett L M (ed.). Wastewater Treatment: Processes, Management Strategies and Envirenmental/Health Impacts. Nova Science Pub Inc, New York, Pg No: 191-213.

11. Hafshejani MK, Ogugbue CJ, Morad N (2014) Application of response surface methodology for optimization of decolorization and mineralization of triazo dye Direct Blue 71 by *Pseudomonas aeruginosa.* 3biotech 4: 605-619.

12. Garg SK, Tripathi M, Lal N (2015) Statistical Design for Optimization of Process Parameters for Biodecolorization of Reactive Orange 4 Azo Dye by *Bacillus cereus* Isolate. Research Journal of Microbiology 10: 502-512.

13. Jadhav SB, Chougule AS, Shah DP, Pereira CS, Jadhav JP (2015) Application of response surface methodology for the optimization of textile effluent biodecolorization and its toxicity perspectives using plant toxicity, plasmid nicking assays. Clean Technologies and Environmental Policy 17: 709-720.

14. Uppala R, Sundar K, Muthukumaran A (2017) Response surface methodology mediated optimization of textile azo dye, Eriochrome Black T decolorization by *Bacillus cereus* RC1. Desalination and Water Treatment 81:242-251.

15. Jaouani A, Neifar M, Prigione V, Ayari A, Sbissi I, et al. (2014) Diversity and Enzymatic Profiling of Halotolerant Micromycetes from Sebkha El Melah, a Saharan Salt Flat in Southern Tunisia. BioMed Research International 14: 439197.

16. El-Hidri D, Guesmi A, Najjari A, Cherif H, Ettoumi B, et al. (2013) Cultivation-dependant assessment, diversity, and ecology of haloalkaliphilic bacteria in arid saline systems of southern Tunisia. BioMed Research International 13: 1-15.

17. Adebajo SO, Balogun SA, Akintokun AK (2017) Decolourization of Vat Dyes by Bacterial Isolates Recovered from Local Textile Mills in Southwest, Nigeria. Microbiology Research Journal International 18: 1-8.

18. Cherif A, Borin S, Rizzi A, Ouzari H, Boudabous A, et al. (2003) *Bacillus anthracis* diverges from related clades of the *Bacillus cereus* group in 16S-23S ribosomal DNA intergenic transcribed spacers containing tRNA Genes. Appl Environ Microbiol 69: 33-40.

19. Si J, Li X-C, Cui B-K (2014) Decolorization of heterocycle dye Neutral Red by white-rot fungus *Perenniporia subacida*. Desalination and Water Treatment 52: 5594–5604.

20. Mathieu D, Nony J, Phan-Tan-Luu R (2000) NEMROD-W Software. LPRAI, Marseille.

21. Nachiyar CV, Rajakumar GS (2003) Degradation of tannery and textile dye Navitan fast blue S5R by *Pseudomonas aeruginosa*. World Journal of Microbiology and Biotechnology 19:609–614.

22. Nachiyar CV, Sunkar S, Kumar GN, Karunya A, Ananth PB, et al. (2012) Biodegradation of Acid Blue 113 Containing Textile Effluent by Constructed Aerobic Bacterial Consortia: Optimization And Mechanism. Journal of Bioremediation and Biodegradation 3: 162.

23. Zabłocka-Godlewska E, Przystaś W, Grabińska-Sota E (2014) Decolourisation of Different Dyes by two *Pseudomonas* Strains Under Various Growth Conditions. Water Air Soil Pollution 225: 1846.

24. Uwidia I E, Ejeomo C (2013) Characterisation of Textile Wastewater Discharges in Nigeria and its Pollution Implications. The Global Journal of Researches in Engineering General Engineering 13: 1-5.

25. Singh D, Narang E, Chutani P, Kumar A, Sharma KK, et al. (2014) Isolation, Characterization and Production of Bacterial Laccase from *Bacillus* sp. In: RN Kharwar, R Upadhyay, N Dubey, R Raghuwanshi (ed.). Microbial Diversity and Biotechnology in Food Security. Springer, New Delhi. Pg No: 439-450.

26. Mishra VK, Sharma H (2014) Decolourization of textile azo dyes by laccase producing *Bacillus* spp. isolated from soil. Asian Journal of Microbiology, Biotechnology and Environmental Sciences 16: 313-319.

27. Neifar M, Jaouani A, Kamoun A, Ghorbel R, Chaabouni S (2011) Decolorization of Solophenyl Red 3BL Polyazo Dye by Laccase-Mediator System: Optimization through Response Surface Methodology. Enzyme Res 2011: 1-9.

28. Shahid M, Muhammad A, Azeem K, Zilli HN, Tariq M (2011) Isolation and screening of azo dye decolorizing bacterial isolates from dye-contaminated textile wastewater. Soil and Environment 30: 7-12: