Open Access

SCHOLARENA

Journal of Environmental Pollution and Management

RESEARCH ARTICLE

ISSN: 2639-7269

Biodecolorization of Azo Dye Reactive Red 220 in Real Textile Wastewater by Active Consortium: Process Optimization Using Mixture Design Model and Toxicity Assessment

Lamia Ayed[1, 2, *], Souhir Torjmèn[1], Neji Ladhari[3] and Kamel Chaieb[1, 4]

[1]*Laboratory of Analysis, Treatment and Valorization of Environmental Pollutants and Products, Faculty of Pharmacy, Route Avicenne, 5000 Monastir, Tunisia*

[2]*Higher Institute of Biotechnology, Monastir (ISBM), 5000 Monastir, Tunisia*

[3]*Higher Institute of Monastir Fashion Trades, Stah Jabeur - Route Korniche, 5000 Monastir, Tunisia*

[4]*Department of Biochemistry, Faculty of Science, King Abdulaziz University, Builiding A 90, Jeddah 21589, Saudi Arabia*

[*]**Corresponding Author:** Lamia Ayed, Laboratory of Analysis, Treatment and Valorization of Environmental Pollutants and Products, Faculty of Pharmacy Monastir, Route Avicenne, 5000, Monastir-Tunisia, Tel.: +21673461000, Fax: +21673461830, E-mail: alym712@yahoo.fr

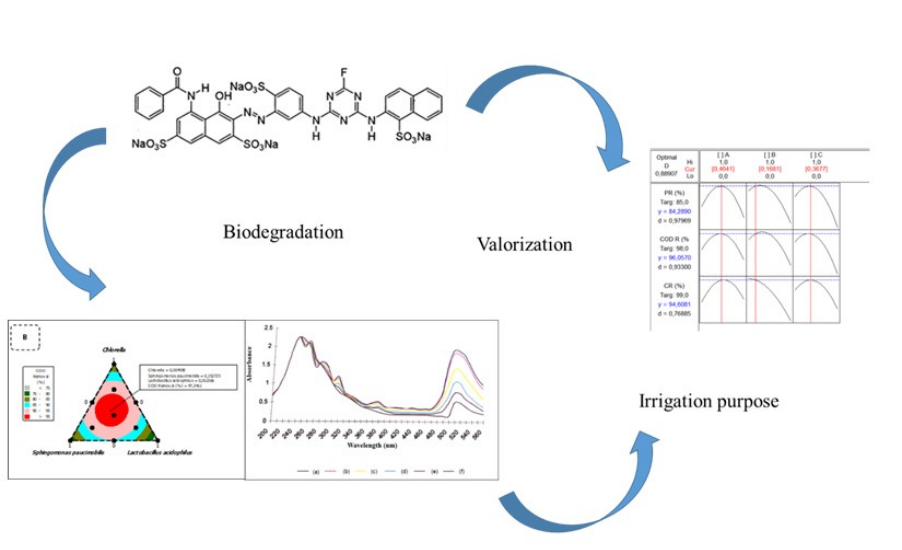
Citation: Lamia Ayed, Souhir Torjmèn, Neji Ladhari, Kamel Chaieb (2024) Biodecolorization of azo dye Reactive Red 220 in Real Textile Wastewater by Active Consortium: Process Optimization Using Mixture Design Model and Toxicity Assessment, J Environ Pollut and Manage 6: 102

Abstract

Azo dyes are an important category of recalcitrant xenobiotics, which are difficult to degrade in cold environments. In this study, three adapted bacteria, isolated from textile wastewater and kefir, were used for developing active consortium able to effective decolorize Reactive Red 220 (CI RR 220) in real textile wastewater (RTW). The most suitable species were Chlorella, Sphingomonas paucimobilis and Lactobacillus acidophilus. Process parameters were optimized using Response Surface Methodology (RSM) and under the optimum conditions (e.g., inoculum size of 10 % (OD600nm =1), 100 rpm, temperature of 25 °C, pH of 11 and time of 5 days). Using batch reactors containing 1000 ppm dye and selected microorganisms, the maximum phenol, COD and color removal efficiencies were found to be 85%, 98 and 99%, respectively. Our results showed that bacteria had a high decolorization capacity. UV-Visible and (FTIR) spectroscopy analysis confirmed the biodegradation and color removal of CIRR 220.

Additionally, the study of cytotoxicity and mutagenicity endpoints, before and after biodegradation of CI RR 220 in real RTW demonstrate the detoxification of reactive azo dye.

Keywords: Reactive Red 220; Response Surface Methodology; Biodegradation, Toxicity; FTIR; UV-Visible



Graphical Abstract

Introduction

Textile wastewater discharged without proper treatment can be yet reactive, toxic and carcinogenic, and can lead to adverse environmental impacts on ecology, agriculture, aquaculture and finally on public human health [1]. Therefore, before this complicated wastewater typology is introduced into a public sanitation network, it must be treated. The process used in the textile business includes turning raw materials into fibers, weaving those fibers into fabrics, applying finishing methods like dyeing or printing, and going through a number of other steps to turn those fibers into completed fabrics. These processes require a lot of energy, chemicals (such dyes and transfer agents), and water [1].

Since textile wastewater (TWW) is characterised by the presence of various contaminants, including synthetic dyes, sulphates and other salts, alkalis, surfactants, dispersing agents, and other organic compounds, which contribute to the high resulting chemical oxygen demand (COD) and dissolved organic carbon (DOC), there are several safety and health issues related to TWW. The majority of dyes used in the textile industry are diazo compounds (70% c.a.), which are distinguished by the -N=N-functional group that links aryl or alkyl groups with an extended electronic conjugation to produce colored molecules [2].

The largest and most diverse class of synthetic dyes, azo dyes, are widely used in the textile, paper and pulp, tannery, pharmaceutical, food, and cosmetic industries [3]. Environmental pollution has been brought about by the increase in industrialization and urbanization, and the expanding market for textile and dyed goods is causing different colors to leak into various natural environments. In addition, because of their low ability to pass through the water's surface, these dyes can have an impact on the aquatic biota by clogging fish gills and stunting the growth of aquatic plants. Difficulties stem from the released wastewater's high temperature, low biodegradability, variable pH, and high color [4,5]. To guarantee the exhaustion step and have a high chemical and photolytic stability, the method uses enormous amounts of salts [6]. According to a number of writers, dyes have the potential to cause serious health problems for humans by mutating, being genotoxic, and being teratogenic. These effects have been linked to problems with the skin, kidneys, digestive tract, brain, liver, and central nervous system [5].

Many physical and chemical methods have been proposed for the removal of dyes from wastewater, including membrane filtration, flocculation, electro-coagulation, silica gel adsorption, and flotation. Nevertheless, the majority of these techniques are costly, time-consuming, produce contaminants, and result in dense sludge that requires additional processing. The microbial treatment of azo dye contaminated areas has drawn increasing attention due to its eco-friendliness, reduced production of hazardous metabo-

lites and sludge, and lower water consumption compared to other alternative approaches [3].

For this reason, creating efficient treatment techniques is essential to advancing water sustainability and environmental preservation. Wastewater from textile dyeing is treated using a variety of technologies and techniques [2,4], however more creative and environmentally friendly techniques are still required. According to Al-Tohamy et al. [7] biological approaches for treating dye effluents are safer, more environmentally friendly, and more promising. Other than bacteria, it has been observed that macroalgae (Cladophora spp., Iridaea cordata, Chara vulgaris) and microalgae (Chlorella vulgaris, Cos-marium spp., Volvox aureus) are potential biological possibilities for treating textile wastewater effluents at significant degrees of decolonization [2]. *Chlorella* sorokiniana was found to be an effective and tolerant strain, utilizing bioaccumulation as the primary mechanism for azo dye removal [8]. Their primary criteria were their capacities for adsorption and biodegradation.

Research and development activities have extensively explored the significance and theoretical underpinnings of RSM as an effective technique for multivariate optimization through sequential experimentation, as well as the optimization through experimental design [9]. Numerous scholars have previously explained the optimization process utilizing a variety of RSM methodologies [4,10].

In this work, real textile effluent from a cotton dyeing and finishing enterprise with high concentrations (1000 ppm) of azo dye CI RR 220 was collected in order to assess the efficacy of an algal-bacterial-probiotic culture. Using RSM, the microalgal-bacterial-probiotic (Chlorella, Sphingomonas paucimobilis and Lactobacillus acidophilus) consortium's performance was assessed in terms of color, phenol, and COD elimination. By comparing metabolites using Fourier-transform infrared spectroscopy (FTIR) analysis and UV-visible spectroscopy before and after treatment, the biodegradation capacity of the chosen consortia was confirmed. The potential toxicity of the produced metabolites was further assessed using mutagenicity and phytotoxicity tests.

Materials and methods

Chemicals

In this investigation, a synthetic reactive dye (CI RR 220; Tianjin Kermel Chemical Reagent Co., Ltd.) was utilized. Using a UV–vis spectrophotometer, the absorbance was determined at the wavelength at which it absorbs lightest (514 nm). The remaining compounds were all analytical grade and of the purest possible purity. The experimental parameters of the extraction process were designed and analyzed using the response surface methodology (Minitab 14.0) [9, 11].

Bacterial Strain, Inoculum Preparation and Screening of the Dye Degrading Microorganism

As previously reported [12], the CI RR 220 degrading bacterium was isolated and screened. For seven days, the bacterial culture that showed the fastest growth was subsequently grown in nutritive broth (NB) medium. 10ml of this inoculum was introduced to real textile wastewater (RTW) solution that had CI RR 220 (1000ppm) and incubated at 100 rpm, temperature of 25 °C and time of 5 days. Three milliliters of the mixture were taken out at regular intervals, centrifuged, filtered, and the absorbance of the mixture was measured at 514 nm using a UV-Vis spectrophotometer. The percentages of color, COD, and phenol elimination were calculated using the following formula (Equation (1), (2), and (3)).

$$color\ \ removal(\%) = \frac{A_{\mathbf{i}} - A_{\mathbf{f}}}{Ai} \times 100 \quad (1)$$

Where A_i was the initial absorbance and A_f was the final absorbance.

$$COD\ \ color\ \ removal(\%) = \frac{COD(0h) - COD(t)}{COD(0h)} \times 100 \quad (2)$$

$$Phenol\ \ removal(\%) = \frac{Phenol(0h) - Phenol(t)}{Phenol(0h)} \times 100 \quad (3)$$

Chlorella, Sphingomonas paucimobilis, and Lactobacillus acidophilus, which form a consortia of microalgae-bacteria, were consistently maintained on Nutrient Agar, which was prepared by combining 1g/L of bacteriological peptone 10, yeast extract (2), beef extract 1, and agar 15. The mixture was then stored at 4 °C until needed. After pre-culturing in nutrient broth prepared as follows (1g/L): peptone 10, NaCl 5, yeast extract 2, and beef extract 1, the selected microorganisms from stock culture were used for biodegradation studies. The cultures were then incubated for 24 hours at 30 ± 2 °C under static conditions and neutral pH [12,13].

Optimization of Microbial CI RR 220-RTW Biodegradation

In order to investigate the region of interest of the parameters discovered by the previous study, response surface methodology (RSM) is typically utilized after a screening study (Ayed et al., 2013). The creation of chemicals, fertilizer, pesticides, food experiments, and other items frequently makes use of mixed designs. In fewer experiment times, it can use regression analysis to determine the link between formulation and performance [9, 11, 14].

For dye change and decolorization indicated as 0% removal, the initial dye absorbance with RTW solution was utilized as a reference.

Fourier Transform Infrared Analysis for Mixed CI RR 220- RTW-Removal Estimation

After the decolorization process was finished, the medium was centrifuged for 25 minutes at 10,000 rpm. With equal quantities of ethyl acetate, CI RR 220 was successfully double extracted in a genuine RTW. In a rotating evaporator, the concentration of isolated metabolites was dried. After the extraction processes were finished, the residue that remained in the flask was dissolved in methanol. Utilizing a UV–visible spectrophotometer, the degree of decolorization was assessed. Then, using FTIR (Perkin-Elmer, Spectrum One) to describe the metabolites produced by dye biodegradation, they were compared to the control dye (the non-degraded one). Using 16 scan speeds, the FTIR analysis was conducted in the mid-IR range of 400–4000 cm-1. Pellets were fixed in a sample holder, samples were combined with spectroscopically pure KBr at a ratio of 2:200, and analysis was performed [11].

Toxicity Assessment of the CI RR 220-RTW Solution Before and After Degradation

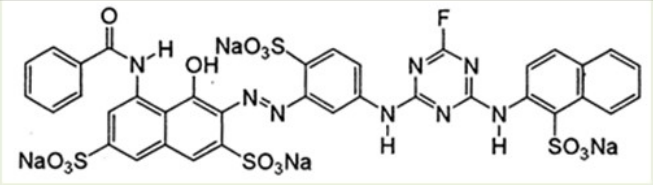
Phytoxicity Assessment

Triticum turgidum ssp. durum seeds were used in a phytotoxicity assay to evaluate the potential toxicity of CI RR 220 in a real RTW and its associated metabolites [2]. The extracted product (dry) and CI RR 220 were independently diluted in distilled water to achieve the desired final concentrations of 1000, 750, 500, and 250 ppm. The 70 seeds of each plant species were grown independently in a control dye solution, and the extracted dye metabolite was used to test the plants' toxicity. After seven days, the percentage of germination of the seed was finally recorded (%). Every test was conducted three times.

Mutagenicity Assessment

Rats administered Aroclor 1254 were used to produce the S9 microsome fraction. 1 milliliter of salt solution, 0.25 milliliters of 1 M G6P, 2 milliliters of 0.1 M NADP, 25 milliliters of 0.2 M sodium phosphate buffer, pH 7.4, 7 milliliters of S9 microsome fraction, and 14.75 milliliters of water made up the S9 mix [15]. Every experiment required a fresh preparation of the S9 mix. The protein BioRad test was used to measure the protein content of rat liver S9 [6]. The measurement was 12.3 mg/mL. The S. Typhimurium TA98 mutagenicity test was carried out in accordance with earlier instructions [6]. In the presence of S.Typhimurium TA98 [16]. For 48 hours, the plates and the revertant bacterial colonies on each plate were incubated at 37° C.

Table 1: Characteristics of CI Reactive Red 220

Parameters	Properties
Color index number	C.I.181065,CAS
Molecular Formula	$C_{36}H_{21}FN_8Na_4O_{14}S_4$
μ_{max} (nm)	514
Structure	

Results and Discussion

Strain Isolation and Identification

Chlorella and Sphingomonas paucimobilis were isolated from raw effluents from a textile wastewater plant located in Ksar Hellal, Tunisia [5, 17] and Lactobacillus acidophilus [9] (Ayed et al., 2019a) was isolated from kefir and selected based on her ability to decolorize CI RR 220 in a real RTW.

Model establishment

Experiment Design and Model Analysis

The experimental values of Lactobacillus acidophilus, Sphingomonas paucimobilis, and Chlorella in decolorized dye utilizing combination starter are shown in Table 2. The regression models for the three responses: color, phenol, and COD removal were created using linear regression fitting. Due to the orthogonality of the design, each effect was evaluated independently in Eqs. (1, 2, and 3). Y stands for the COD, phenol, and color removal. RSM is typically used in accordance with screening studies to investigate the factors that the previous study indicated in the interest region [4, 18]. Regression analysis using the mixed design can determine the relationship between formulation and performance in fewer trial times.

Y (DCO (%)) = 79.49 S1 + 75.86 S2 + 70. 31 S3 + 63.15 S1*S2 + 68.06 S1*S3 + 64.79 S2*S3; (Eq1) R2=89.12 ; p=0.046

Y (Phenol (%)) = 59.20 S1 + 54.47 S2 + 51.11 S3 + 91.86 S1*S2 + 101.13 S1*S3 + 71.68 S2*S3; (Eq2) R2=89.49 ; p=0.043

Y (Decolorization (%)) = 86.62 S1 +77.71 S2 + 82.62 S3 + 32.28 S1*S2 + 42.10 S1*S3+ 20.28 S2*S3; (Eq3) R2=38.92 ; p=0.761

Were S1: Chlorella, S2: Sphingomonas paucimobilis and S3: Lactobacillus acidophilus

CI RR 220	Germination	Root length (cm)	Shoot length (cm)
Control	1000	4.40.2	6.20.1
BT 25%	250.4	0.70.1	1.80.1
BT 50%	200.3	0.50.1	1.50.2
BT75%	150.3	0.40.01	1.20.1
BT 100%	100.2	0.20.01	0.90.01
AT 25%	1000.1	3.90.2	6.10.4

AT 50%	1000.1	3.70.1	5.80.3
AT 75%	1000.1	3.50.1	5.50.2
AT 100%	980.2	3.40.1	4.90.1

Table 2: Mixture design matrix with the experimental analysis

The study's R2 values were 0.8912, 0.8949, and 0.3892, indicating that the independent variables account for 89.12%, 89.49%, and 38.92% of the total variations, while the model for COD, phenol, and color removal only explains 10.88%, 10.51%, and 61.08% of the changes overall. When a high value of R2 was attained, Altayb and colleagues [10] claim that the generated model can provide a good assessment for the response within the range of process circumstances. The Fisher F-test (Table 3) in conjunction with analysis of variance (ANOVA) was used to confirm the model's sufficiency and significance The F-value is the mean square of the regression over the mean square of the residual. The mean square is computed by dividing the sum of the squares of the error variance and the model variance by the corresponding degree of freedom. Ayed et al. [19] state that the computed F-value must be higher than the tabulated F-value from the standard distribution table in order to support the model's efficacy based on the experimental data [19]. Table 3 displays the ANOVA model for the COD and color removal with a high confidence level of 95%. According to the model F-value, the regression equation accounts for the majority of the variation in the answer. To determine if the F-ratio is large enough to suggest statistical significance, one uses the related P-value. If the P-value is more than 0.1, meaning that $\alpha = 0.05$ or 95% confidence level is reached, the model cannot be deemed statistically significant. The quadratic model is statistically significant for the response, as indicated by the non-significant value of lack of fit (>0.05), and as such, it can be employed for additional analysis [4, 9].

Assay	***Chlorella* (S1)**	***Sphingomonas paucimobilis* (S2)**	***Lactobacillus acidophilus* (S3)**	**Total**	**Phenol Removal (%)**	**COD Removal (%)**	**Color Removal (%)**
1	1	0	0	1	60	80	85
2	0	0	1	1	54	76	83
3	0	1	0	1	51	70	81
4	0.33	0.33	0.33	1	75	90	92
5	0.5	0.5	0	1	76	88	90
6	0.5	0	0.5	1	65	85	87
7	0	0.5	0.5	1	**85**	**98**	**99**
8	0.16	0.66	0.16	1	83	96	98
9	0.66	0.16	0.16	1	82	95	97
10	0.16	0.16	0.66	1	80	94	95

Table 3: Analysis of Variance for color, COD and phenol Removal (%)

DF: Degrees of Freedom; Seq SS: Sequential Sums of Squares; Adj MS: Adjusted Mean Squares ; F-ratio: Analysis of Variance table ; P-value: Probability

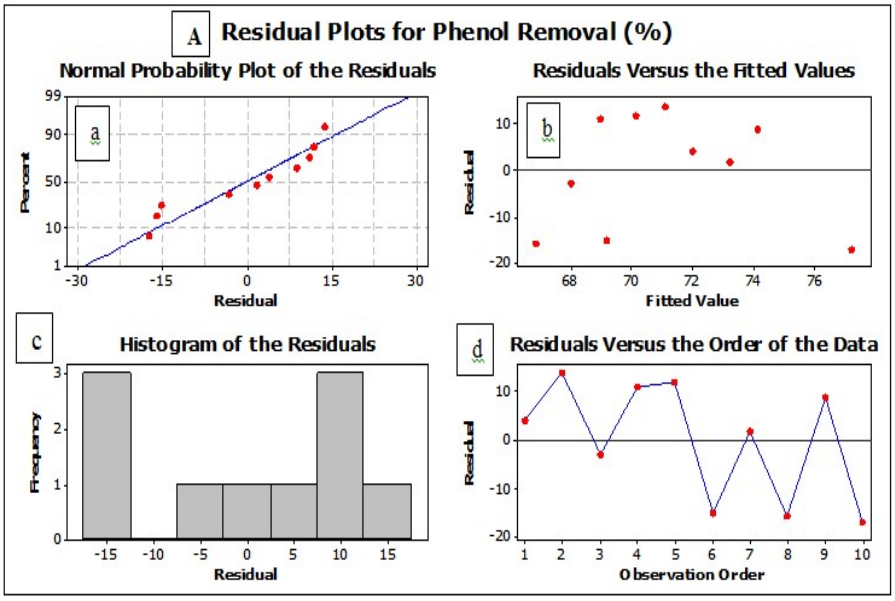
Interpretation of Residual Plot

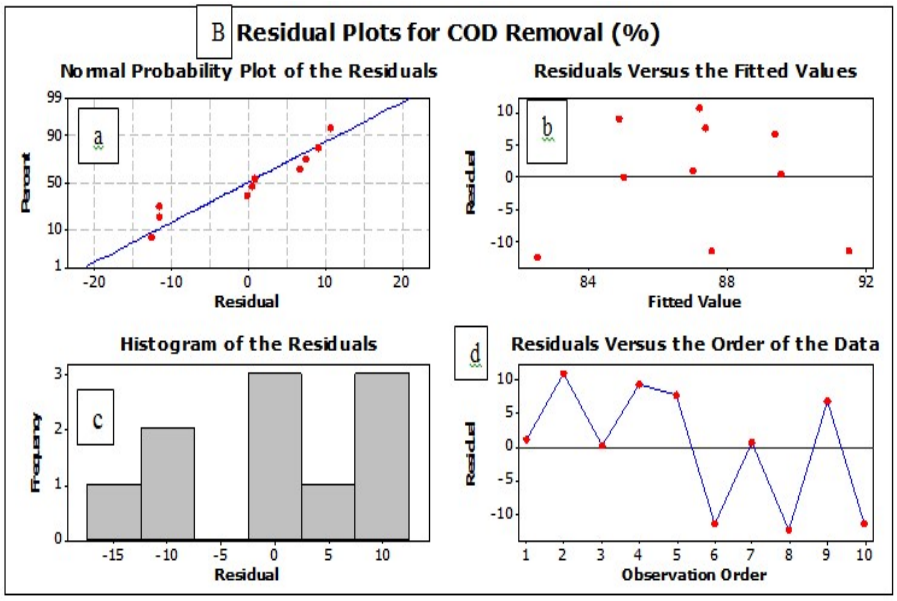
The residual values can then be plotted in a normal probability plot for phenol (Figure 1A(a)), COD (Figure 1B(a)) and color removal (Figure 1C(a)). All points from this residual plot lies close to the straight line confirming the conjecture that effects other than those considered in the model may be readily explained by random [20]. The experimental values were scattered randomly within a constant range of residuals across the graph, which indicated that the proposed model and constant variance assumption were suitable [5].

Also, plotting externally studentized residuals versus an experimental runs plot shows that all the data points lay within the limits, which indicated satisfactory fit of the developed model for phenol (Figure 1A(b)), COD (Figure 1B(b)) and color (Figure 1C (b)) removal.

The histogram of the residual (Figure 1A(c); 1B(c) and 1C (c)) can be used to check whether the variance is normally distributed [4]. A symmetric bell shaped histogram which is evenly distributed around zero indicates that the normality assumption is likely to be true. In addition, the correlation between the predicted and actual values is shown in in which the points were close to the diagonal line and implied low discrepancies between them [9].

Also, plotting externally residuals versus an experimental runs plot (Figure 1A(d) ; 1B(d) and 1C(d),), shows that all the data points lay within the limits, which indicated satisfactory fit of the developed model [21]. The experimental values were scattered randomly within a constant range of residuals across the graph, which indicated that the proposed model and constant variance assumption were suitable.





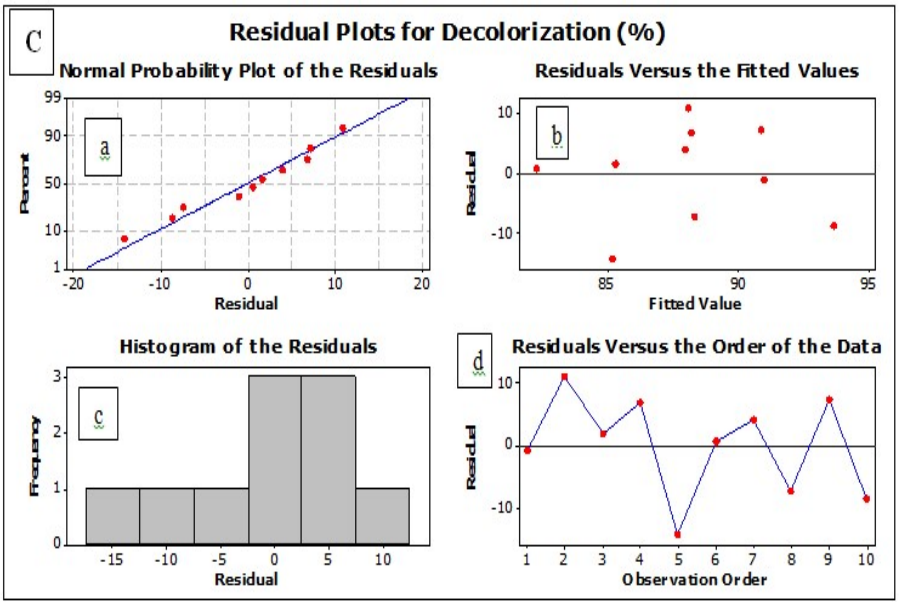


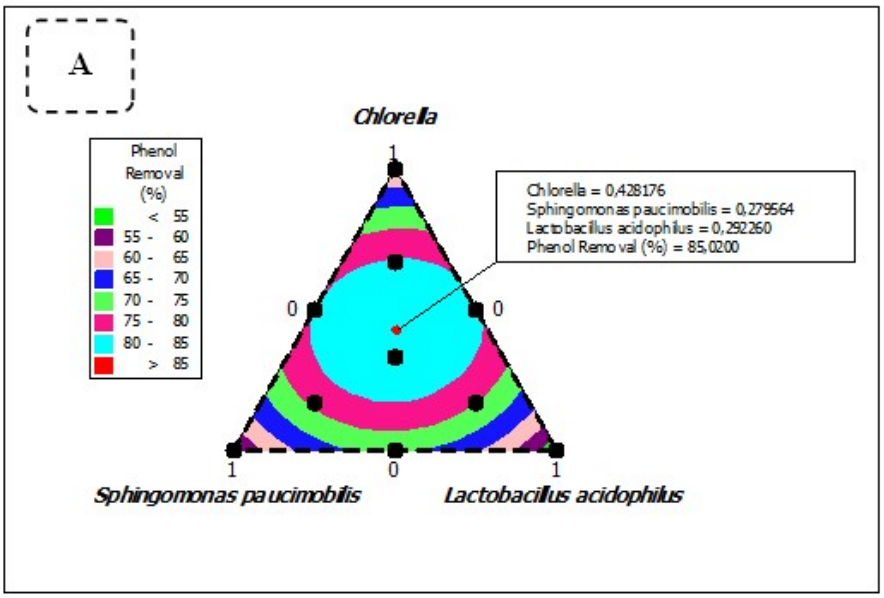
Figure 1: Residual plots for Phenol Removal (A), COD Removal (B) and Color Removal (C) CI RR 220 in Real TWW

Interpretation of Contour Plot

In order to confirm the phenol 85%, 98 % COD and 99 % color removal obtained experimental results; a contour plot (Figure 2) was plotted. The higher phenol (85.02%), COD (97.24%) and color removal (95.23%) yields were obtained when Chlorella, Sphingomonas paucimobilis, and Lactobacillus acidophilus proportions were (42.81%, 27.95 % and 29.22 %); (38.49%, 35.27 % and 26.23 %) and (55.38%, 0.082 % and 44.53 %, respectively for phenol, COD and color removal.

The mixture surface plot also described individual and cumulative effect of these three variables and their subsequent effect on the response [22].

Additionally, Dafale and collaborators [23] showed that 70% COD was removed during shaking condition for Remazol Black B (RBB) [23]. Similarly, Jadhav and collaborators [24] indicated that Galactomyces geotrichum MTCC 1360 was able to decolorize Methyl Red (100%), Malachite green (97%), Scarlet RR (100%), Orange HE 4B (75%) and Amido black 10B (92%) in malt yeast medium with shaking condition [24].



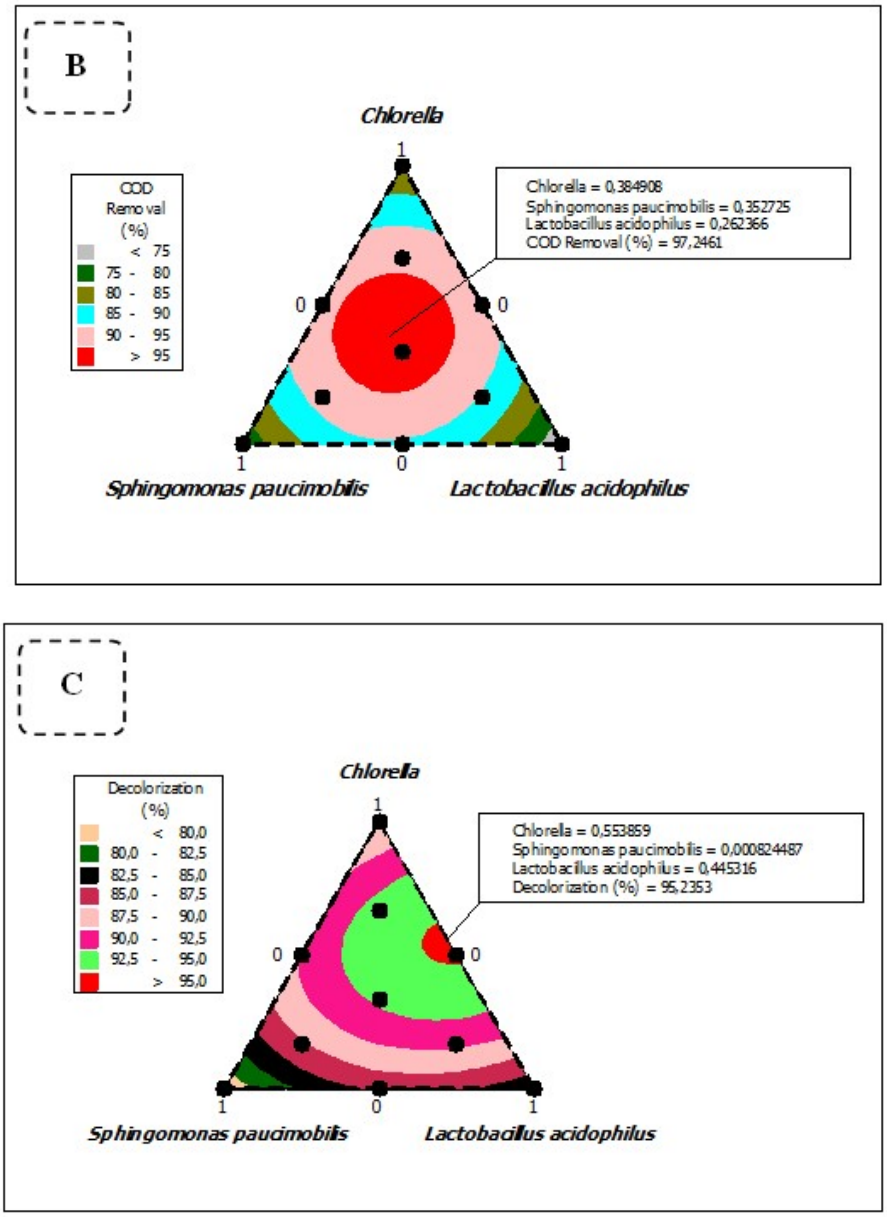


Figure 2: Mixture Contour plots for phenol (A), COD (B) and color removal (c) of CI Reactive Red 220 in Real TWW by Chlorella, Sphingomonas paucimobilis and Lactobacillus acidophilus

Optimization Plot to Confirm the Experimental Results

In order to confirm the experimental results that 85% phenol, 98 % COD and 99 % color removal a response overlaid contour (Figure 3) was plotted by MINITAB. 14 Software Programme where minimum maximum values percentage of phenol, COD and color removal which were fixed (60–100%), (70-99%) and (50–90%). The results thus obtained and were predicted if the Chlorella proportion was 46.41%, Sphingomonas paucimobilis was 16.81% and Lactobacillus acidophilus was 16.81%; to remove 94.60% of color of dye, 96.05% of COD and 84.28% of phenol. The application of optimization plot effectively alleviated the COD, color and phenol limitation of microbial resources.

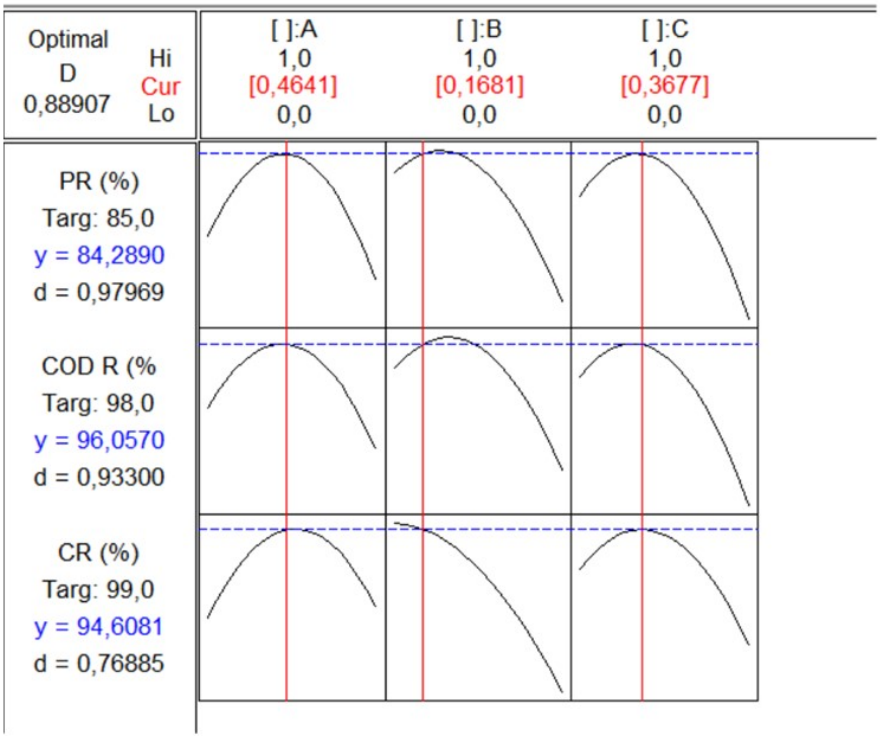


Figure 3: Optimization plot to confirm the experimental results that 94.60% CR (Color Removal, 96.05% COD R (removal) and 84.28% PR (Phenol removal) when the consortium composition was 46.41% Chlorella (A), Sphingomonas paucimobilis 16.81% (B) and 36.77%, Lactobacillus acidophilus (C).

FTIR and UV-Visible Spectroscopy Analysis for Adsorption and Removal of CI RR 220-RTW

To understand the possible way of decolorization of CI RR 220 in real RTW, the supernatant was analyzed by UV-visible spectrophotometer and FTIR. Biodegradation of CI RR 220 by selected organisms was confirmed by FTIR spectroscopic analysis (Figure 4a and 4b). Biodegradation is indicated either by disappearance of absorbance peaks or appearance of new peaks [25]. The FTIR spectrum of the dye before biodegradation represented H–bonded of alcohols and phenols at 3480 cm -1 , S=O at 1100 cm-1 and C-N at 1620 cm-1. O–H stretch, H–bonded of alcohols and phenols is a very broad and strong band which took an active part in the adsorption of CI RR 220 because of the presence of hydrogen bonding. The FTIR spectrum of 5days extracted metabolites showed a significant change in the bands positions compared to control dye spectrum. The intensities of band at 3480 cm^{-1}, 1620 cm^{-1} and 1100 cm^{-1} was decreased and a new band observed at 850 cm-1 represented C–H deformation of alicyclic CH2, whereas another band were disappeared, it represents a response of an out-of-plane deformation of a hydrogen atom linked to an aromatic cycle and the decomposition of the azo group and formation of aromatic amines which are of low basicity. The disappearance of absorption peaks and appearance of new peaks in the FTIR spectrum were reported due to biodegradation of CI RR 220.

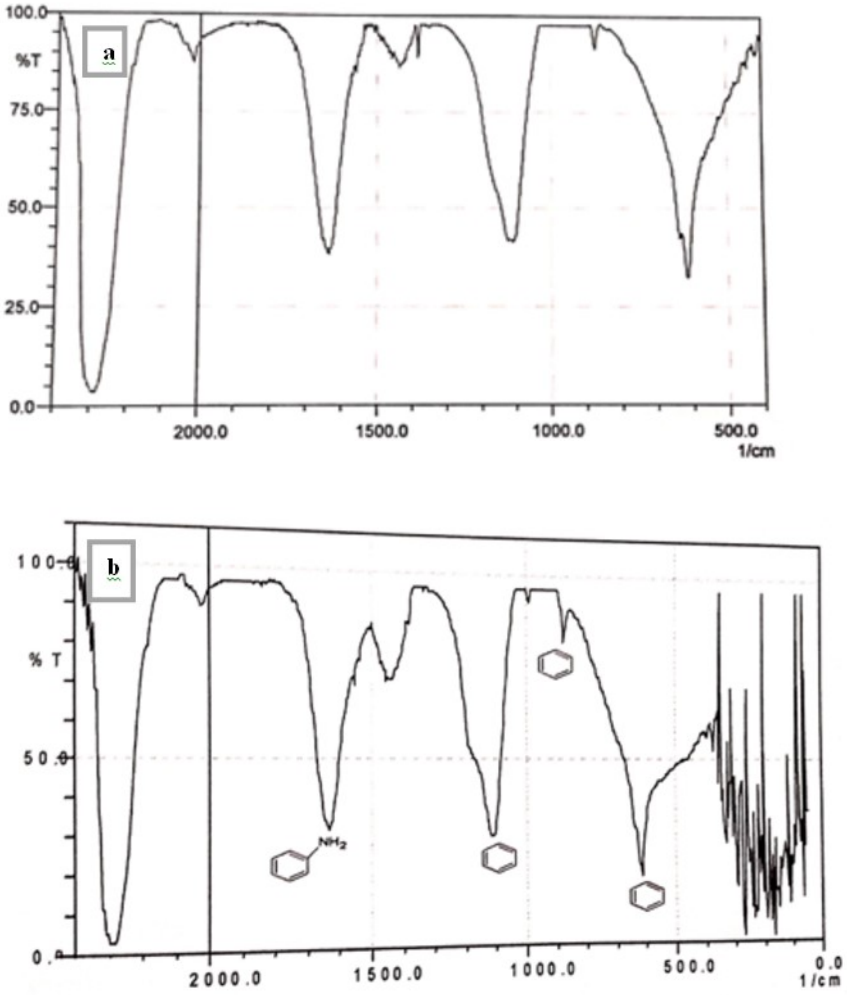


Figure 4: FTIR spectra of CI Reactive Red 220 before (a) and after degradation (b) under optimized condition in effluent textile wastewater.

Changes in the UV–Vis absorption spectrum (e.g., from 200 to 500 nm) were observed for decolorization of CI RR 220 and compared to the respective control samples. Dyes adsorption on the surface of bacterial cells or biodegradation is primarily responsible for the bacteria assisted de-colorization of dyes [26]. For dye adsorption, the peaks in the UV–Vis absorption spectrum diminish almost in a proportional ratio to each other. However, in case of biodegradation of dyes, either the crucial absorbance peaks in the UV-visible region completely vanished or new peaks popped up. The UV–Vis absorption spectrum (200–600 nm) (Figure 5) of CI RR 220 dye showed a shift in λmax (514nm) of the control to a shorter wavelength (258 nm) upon complete decolourization, demonstrating the formation of new pic at 375nm, 314nm and 284nm in the supernatant of culture media as a consequence of dye biodegradation. Figure 5 shows the UV–vis absorption spectra of the initial solutions and the decolorized samples of CI RR 220 in real RTW from the SBR. As the biodegradation proceeded (5 days), the peak at 514 nm disappeared. The disappearance in absorbance at 514 nm is likely due to the degradation of the CI RR 220 chromophore [27], and the degradation is constant in one cycle. The shift in light absorbance spectra is attributed to new metabolites due to biodegradation of the parent compound [25, 28, 29].

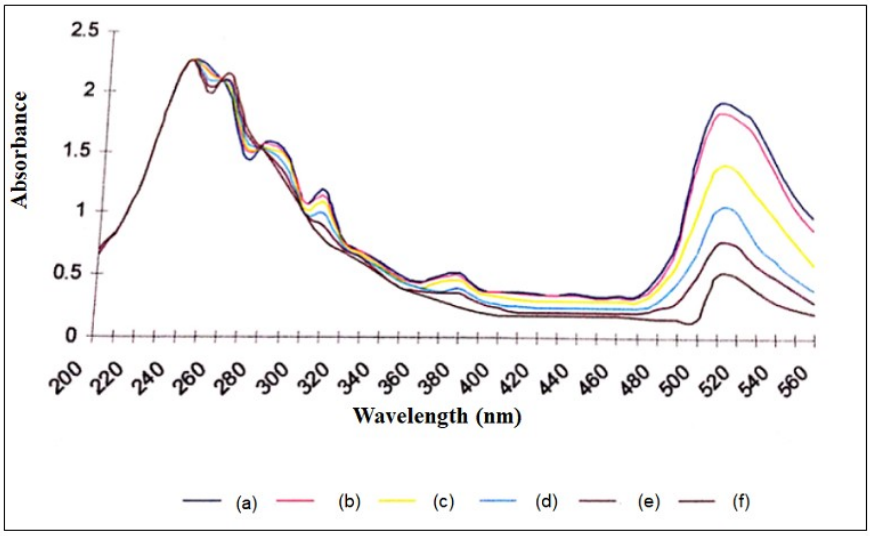


Figure 5: UV–vis spectra for CI Reactive Red 220 by Chlorella, Sphingomonas paucimobilis and Lactobacillus acidophilus in effluent textile wastewater (a: before biodegradation; b: 1 day ; c: 2 days ; d: 3 days ; e: 4 days and f : 5 days).

Phytotoxicity of the Treated CI RR 220 in RTW

The reuse of treated wastewaters in agricultural activities can be a feasible solution to tackle water scarcity in water-stressed regions of the world. Unfortunately, few studies in the literature have systematically assessed the potential of treated textile wastewaters for irrigation or the effects over soil chemical composition and plant growth. The unexpectedly superior growth of Triticum turgidum ssp. durum when irrigated with RTW may be attributed to the high macronutrients and micronutrients content of the raw wastewater compared to tap water. These results highlighted the potential reuse of treated water for plant irrigation. Finally, it is worth noting that the high Triticum turgidum ssp. durum dry weight, and area and number of leaves, recorded when irrigated with RTW, and the lack of difference in plant physiology when compared to the plants irrigated with treated water, might be due to the limited duration of the experiments conducted. Thus, it was of concern to assess the phytotoxicity (Table 4) of the dye CI RR 220 before (germination 0%) and after degradation. The control test was the distilled water (germination 100%). Plant seed germination and early seedling growth test have been considered as one of the simplest and short-term methods for the study of the general toxicity of the chemicals and industrial wastewaters [6]. The phytotoxicity indicated a no toxicity of the degradation products to the plants. Similarly we have showed that the percentage of germination of Triticum aestivum was less using Malachite Green and Crystal Violet solution compared to its degraded products [12]. Hence phytotoxicity studies revealed that the biodegradation of wastewater containing dyes by a microbial culture resulted by its complete detoxification. These treated effluents can be used for ferti-irrigation. However these findings suggested the non-toxic nature of the formed products. Previous works showed that the Malachite Green and Crystal Violet degradation into leucomalachite and leucocrystal violet are equally toxic to the initial compound [30].

Source	DF	Seq SS	Adj MS	F-ratio	P-value
Color Removal					
Regression	5	257.969	51.594	0.51	0.761
Residual Error	4	404.931	101.233		
Total	9	662.900			
COD Removal					
Regression	5	701.903	140.381	6.55	0.046

Residual Error	4	85.697	21.424		
Total	9	787.600			
Phenol Removal					
Regression	5	1278.69	255.737	6.81	0.043
Residual Error	4	150.21	37.553		
Total	9	1428.90	352.500		

Table 4: Effect of different concentration of CI RR 220 in RTW before (BT) and after (AT) biodegradation on seed germination, root length, shoot length of early seedling of Triticum turgidum ssp. durum

Values are mean ± SD (n = 3).

Mutagenicity Assessment

Generally, a compound is classified as mutagen if it is able to increase at least twice the number of revertants compared to spontaneous revertant [16]. We reported in this investigation that after biodegradation of CI RR 220 the mutagenicity decreased when compared to the untreated pure dye even with the S9 metabolizing system. The revertant number exceeds 2-folds the spontaneous revertants in Salmonella Typhimurium TA98 assay systems at the highest tested dose of 250 g/assay. However, we noticed no mutagenicity of CIRR220 products obtained after biodegradation with optimised consortium (46.41% Chlorella, 16.81% Sphingomonas paucimobilis and 16.81% Lactobacillus acidophilus) with the S9 metabolizing system (Table 5). According to Ben Mansour and collaborators [15] the absence of mutagenic effect of azo dye AV7 metabolites from shaken cultures with Pseudomonas putida mt-2 could be explained by the presence of a sufficient level of dissolved oxygen that might limit the azoreductase action and increase the oxygenase activity, allowing the degradation of the dye aromatic amines to which may be ascribed the mutagenic effects [15].

CI RR 220	Germination	Root length (cm)	Shoot length (cm)
Control	1000	4.40.2	6.20.1
BT 25%	250.4	0.70.1	1.80.1
BT 50%	200.3	0.50.1	1.50.2
BT75%	150.3	0.40.01	1.20.1
BT 100%	100.2	0.20.01	0.90.01
AT 25%	1000.1	3.90.2	6.10.4
AT 50%	1000.1	3.70.1	5.80.3
AT 75%	1000.1	3.50.1	5.50.2
AT 100%	980.2	3.40.1	4.90.1

Table 5: Mutagenic activity of CI RR 220 in RTW, before and after biodegradation evaluated by the Ames and assay using Salmonella typhimurium TA98 in the presence of S9 mix

Positive control (PC : Nitro-2-Fluoren), SR: spontaneous revertant.

Conclusion

Our study proved the ability of Chlorella, Sphingomonas paucimobilis and Lactobacillus acidophilus to degrade and detoxify the industrial reactive azo dye CI RR220 in real textile wastewater, and displayed their potential uses in degradation of textile dye and converts non toxic products to the environment. The phytotxicity experiments demonstrated a superior detoxification of CI RR220 by this consortium. This study was conclude the mutagenicity and phytotoxicity of the intact dye and degraded products of

dye demonstrate that the consortium interceded degradation prompts to the development of non toxic intermediates affirmed Ames [16] toxicity assays.

The competence of the bacterial consortium to degrade high concentrations of reactive dyes and convert them into non toxic byproducts for plants, human and aquatic life forms attracts Chlorella, Sphingomonas paucimobilis and Lactobacillus acidophilus as a potential micro-organism for bioremediation of textile dyes. These results indicate that the bacteria would represent a promising biotechnological tool for the biodegradation of environments co-contaminated with textile wastewater.

Highlights

- Biodecolorization and degradation of Reactive Red 220 dye in real TTW by bacterial strain
- Optimization of process parameters using RSM based mixture design model
- At optimized conditions, about 99% dye decolorization was achieved within 5days.
- Biodegradation of dye was established by various analytical approaches.
- Degraded dye showed less genotoxic and phytotoxic effects.

Declaration of Interests

Disclosure of potential conflicts of interest

The authors declare no conflict of interest.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Research involving Human Participants and/or Animals

- Not Applicable

Informed Consent

Not Applicable

Ethical Approval

Not Applicable

References

1. Jallouli S, Buonerba A, Borea L, Hasan SW, Belgiorno V et al. (2023) Living membrane bioreactor for highly effective and eco-friendly treatment of textile wastewater. Science of the Total Environment, 871: 161963.

2. Chaieb K, Kouidhi B, Ayed L, Bakr HS, Abdulhakim JA et al. (2023) Enhanced textile dye removal from wastewater using natural biosorbent and Shewanella algae B29: Application of Box Behnken design and genomic approach. Bioresource Technology, 374: 128755.

3. Alghamdi MA, Ayed L, Aljarad MR, Altayeb HN, Abbes S et al. (2023) Whole genome sequencing analysis and Box-Behnken design for the optimization of the decolourization of mixture textile dyes by halotolerant microbial consortium. Microbiological Research, 276: 127481.

4. Ayed L, Ksibi IE, Charef A, Mzoughi RE (2020) Hybrid coagulation-flocculation and anaerobic-aerobic biological treatment for industrial textile wastewater: pilot case study. The Journal of the Textile Institute: 1-7.

5. Ayed L, Zmantar T, Bayar S, Cheref A, Achour S et al. (2019b) Potential use of probiotic consortium isolated from kefir for textile azo dye decolorization. Journal of microbiology and biotechnology, 29: 1629-5.

6. Ayed L, Bakir K, Mansour HB, Hammami S, Cheref A et al. (2017a) 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. Microbial pathogenesis, 103: 29-39.

7. Al-Tohamy R, Ali SS, Li F, Okasha KM, Mahmoud YAG et al. (2022) A critical review on the treatment of dye-containing wastewater: Ecotoxicological and health concerns of textile dyes and possible remediation approaches for environmental safety. Ecotoxicology and Environmental Safety 231: 113160.

8. Tarbajova V, Kolackova M, Chaloupsky P, Dobesova M, Capal P et al. (2023) Physiological and transcriptome profiling of Chlorella sorokiniana: A study on azo dye wastewater decolorization. Journal of Hazardous Materials, 460: 132450.

9. Ayed L, Bouguerra A, Charef A, Bakhrouf A, Mzoughi RE (2019a) Biodegradation of Olive Mill Wastewater by a newly isolated novel bacterial consortium under RSM optimized culture conditions. Journal of Water Process Engineering, 32: 100986.

10. Altayb HN, Kouidhi B, Baothman OA, Abdulhakim JA, Ayed L et al. (2021) Mathematical modeling and optimization by the application of full factorial design and response surface methodology approach for decolourization of dyes by a newly isolated Photobacterium ganghwense. Journal of Water Process Engineering, 44: 102429.

11. Ayed L, Chakroun I, Zmantar T, Jabeur C (2023) The Use of Sugarcane Bagasse and Yeast Extract as Agro-Industrial Coproducts for Bioremediation Strategies of Paper Mill Wastewater Using Pseudomonas putida. Water Air and Soil Pollution, 234: 470.

12. 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. Chemical Engineering Journal, 162: 495-502.

13. Ayed L, Chaieb K, Cheref A, Bakhrouf A (2009) Biodegradation of triphenylmethane dye Malachite Green by Sphingomonas paucimobilis. World Journal of Microbiology and Biotechnology, 25: 705.

14. Ayed L, Bochra K, Karima B, Amina B (2013) Biodegradation of azo and triphenylmethanes dyes: cytotoxicity of dyes, slime production and enzymatic activities of Staphylococcus epidermidis isolated from industrial wastewater. African Journal of Microbi-

ology Research, 7: 5550-7.

15. Ben Mansour H, Mosrati R, Corroler D, Ghedira K, Barillier D, Chekir L (2009) In vitro mutagenicity of Acid Violet 7 and its degradation products by Pseudomonas putida mt-2: correlation with chemical structures. Environmental toxicology and pharmacology, 27: 231-6.

16. Ames BN, McCann J, Yamasaki E (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Research/Environmental Mutagenesis and Related Subjects, 31: 347-63.

17. Cheriaa J, Bettaieb F, Denden I, Bakhrouf A (2009) Characterization of new algae isolated from textile wastewater plant. Journal of Food, Agriculture and Environment 7: 700-4.

18. 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. African Journal of Biotechnology, 1: 12339-55.

19. Ayed L, Bekir K, Jabeur C (2022) Modelling and optimization of biodegradation of methylene blue by Staphylococcus aureus through a statistical optimization process: A sustainable approach for waste management. Water Science and Technology, 86: 380-94.

20. Tzikalos N, Belessi V, Lambropoulou D (2013) Photocatalytic degradation of Reactive Red 195 using anatase/brookite TiO 2 mesoporous nanoparticles: optimization using response surface methodology (RSM) and kinetics studies. Environmental Science and Pollution Research, 20: 2305-20.

21. Ghasemi M, Ghoreishian SM, Norouzi M, Badii K, Ozaee K et al. (2018) Optimization of sonophotocatalytic decolorization of Begazol Black B by loaded, double-sided nanophotocatalysts on porous substrate: a central composite design approach. Journal of the Taiwan Institute of Chemical Engineers, 93: 166-75.

22. Mnif I, Bouassida M, Ayed L, Ghribi D (2023) Optimization of textile effluent bacterial treatment and improvement of the process efficiency through SPB1 biosurfactant addition. Water Science & Technology 87: 1764-78.

23. Dafale N, Wate S, Meshram S, Nandy T (2008) Kinetic study approach of remazol black-B use for the development of two-stage anoxic–oxic reactor for decolorization/biodegradation of azo dyes by activated bacterial consortium. Journal of hazardous materials, 159: 319-28.

24. Jadhav S, Kalme S, Govindwar SP (2008) Biodegradation of methyl red by Galactomyces geotrichum MTCC 1360. International Biodeterioration & Biodegradation, 62: 135-42.

25. Sen SK, Patra P, Das CR, Raut S, Raut S (2019) Pilot-scale evaluation of bio-decolorization and biodegradation of reactive textile wastewater: an impact on its use in irrigation of wheat crop. Water Resources and Industry, 21: 100106.

26. Asad M, Brahim M, Ziegler-Devin I, Boussetta N, Brosse N (2017) Chemical Characterization of Non-Saccharidic and Saccharidic Components of Rapeseed Hulls and Sunflower Shells. BioResources, 12: 3143-53.

27. Liu W, Liu L, Liu C, Hao Y, Yang H et al. (2016) Methylene blue enhances the anaerobic decolorization and detoxication of azo dye by Shewanella onediensis MR-1. Biochemical Engineering Journal, 110: 115-24.

28. Pandey A, Singh P, Iyengar L (2023) Bacterial decolorization and degradation of azo dyes. International Biodeterioration & Biodegradation, 59: 73-84.

29. Ajaz M, Shakeel S, Rehman A (2020) Microbial use for azo dye degradation—a strategy for dye bioremediation. International Microbiology, 23: 149-59.

30. Ayed L, Bekir K, Achour S, Cheref A, Bakhrouf A (2017b) Exploring bioaugmentation strategies for azo dye CI Reactive Violet 5 decolourization using bacterial mixture: dye response surface methodology. Water and Environment Journal, 31: 80-9.