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Response surface methodology for textile wastewater decolourization and biodegradation by a novel mixed bacterial consortium developed via mixture design

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ABSTRACT

The present study clearly showed the existence of good scope for the bacterial consortium composed of *Sphingomonas paucimobilis*, *Bacillus* sp. and *Staphylococcus epidermidis* in the decolourization of textile wastewater. The full factorial plan method was used for the multiobjective optimization of bacteria with the decolourization process as response to explore a new optimization method of the mixed starter. The influence of temperature, agitation and pH on colour and chemical oxygen demand (COD) was investigated using 2^3 factorial designs. The contours curve generated by the software MINITAB corresponding to the manipulation at a fixed temperature of 28°C shows the optimal conditions for the decolourization which are: temperature 28°C, pH close to neutrality (7.2–7.25) and a restlessness that ranges from 100 to 150 rpm. The decolourization and the biodegradation yields increased with the developed consortium with yields of 100 and 75.76% of colour and COD removal, respectively.

Keywords: Experimental Design; Decolourization; Biodegradation; Bacterial consortium; Mixture design

1. Introduction

Wastewater generated by different production steps of a textile mill has high pH, temperature, detergents, oil, suspended and dissolved solids, toxic and non-biodegradable matter, colour and alkalinity and high chemical oxygen demand (COD) [1]. The textile industry wastewater is rated as the most polluting among all industrial sectors considering both the volume and the effluent composition [2]. It has been proven that some of these dyes and/or products are carcinogens and mutagens [3]. Those chemicals are discharged into wastewater in different process stages of textile industry. So, textile wastewater containing

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dyes must be treated before their discharge into the environment [4].

Physicochemical methods are applied for the treatment of this wastewater, achieving high dye removal efficiency [5]. Numerous processes have been proposed for the treatment of coloured wastewater e. g. precipitation, flocculation, coagulation, adsorption and wet oxidation [6,7]. All these methods have different colour removal capabilities, capital costs and operating speed. Among low cost, viable alternatives, available for effluent treatment and decolourization, biological systems are characterized by their capacity to reduce the biochemical oxygen demand and COD by conventional aerobic biodegradation [8,9].

Recently, there has been a tendency to use biological treatment systems to treat dye-bearing wastewater, and aerobic processes have been used for the treatment of textile wastewater as stand-alone processes [2] and it is confirmed that they are efficient; cost-effective for smaller molecules [10]. Many micro-organisms belonging to different taxonomic groups of bacteria [11,12] actinomycetes, [13] and algae [14] have been reported for their ability to decolourize these wastes. Although many reports are available in the literature regarding the capability of pure cultures to decolourize textile wastewater containing dyes [15], they do not find many applications in treatment systems for industrial effluent because of the existence of heterogeneous components in effluents depending upon production schedule. The treatment systems having mixed microbial populations are more effective due to concerted metabolic activities of microbial community [16]. Nowadays, bacterial strains displaying substantial growth in aerobic culture have been described [17]. Mixed culture studies may be more appropriate for the decolourization of dyes.

In this way, the factorial experimental design, which involved changing all the variables from one experiment to the next, was chosen in order to estimate the influence of the different variables. Factorial designs are widely used to investigate the effects of experimental factors and the interactions between those factors; that is, how the effect of one factor varies with the level of the other factors in a response. The advantages of factorial experiments include the relatively low cost, a reduced number of experiments and increased possibilities to evaluate interactions among the variables. The most popular first-order design is the two-level full (or fractional) factorial, in which each factor is experimentally studied at only two levels that are expressed in coded form: (−1): low level and (+1): high level. The full factorial design consists of a 2^k experiment (k factors, each experiment at two levels), which is extremely useful for either preliminary studies or in initial optimization steps, while fractional designs are almost mandatory when the problem involves a large number of factors [18,19].

This work aimed at optimizing the treatment of textile wastewater by a novel bacterial consortium (*S. paucimobilis*, *Bacillus* sp. and *S. epidermidis*) using custom response surface methodology (RSM). In fact, when we select optimal conditions for the growth and the different proportions of these three micro-organisms using the RSM, we can ameliorate the decolourization and biodegradations performances of the cells for the textile wastewater.

2. Materials and methods

2.1. Microbial strains

This study investigated the combined effects of three species *S. paucimobilis*, *Bacillus* sp. and *S. epidermidis* on colour and COD removal of textile wastewater to understand their roles in organic removal and how they interacted with each other. These bacterial strains were previously isolated by Ayed et al. [20–22] using culture enrichment techniques from the aerobic sludge of a plant treating textile wastewater. These micro-organisms are deposited in the culture collection of the Laboratory of Analysis, Treatment and Valorization of Environmental Pollutants and Products (Tunisia). The culture was maintained at 4 °C in nutrient agar slants as working stock cultures.

2.2. Acclimatization

The acclimatization was performed by gradually exposing bacteria to increasing concentrations of the effluent. *S. paucimobilis*, *Bacillus* sp. and *S. epidermidis* were transferred into the nutrient medium. The used medium was composed of 1,000 mL of textile wastewater (COD = 4,300 mg/L, colour = 1,346 Colour Unit (CU)): glucose (1,250 mg/L) and yeast extract (3,000 mg/L) [23].

For study use, aerobic growth experiments were conducted in batch cultures. The strains were inoculated with total inocula of 10% v/v. A loopful of growth form stock culture slope was inoculated into the nutrient medium. After 24 h of incubation, the culture broth (10 mL) was transferred in triplicates in 250 mL flasks containing 90 mL of the twice-diluted effluent (total volume 100 mL). The pH of the medium was adjusted to the desired values ranging between 7.2 and 8.5 according to the chosen level of the factorial design. After inoculation, the flasks were incubated at

the corresponding temperatures (28 and 37°C) and shaking conditions (100 and 150 rpm). The uninoculated control was also incubated to check the abiotic decolourization.

2.3. Analytical methods

Samples were centrifuged at 6,000 rpm for 10 min and analysed for colour, COD and pH. COD and colour measurements were carried out on the clear supernatant. Colour was measured by an UV–vis spectrophotometer (UV–vis Double Beam PC Scanning spectrophotometer UVD-2960) at a wavelength of 675 nm in which maximum absorbance spectra was obtained for the dye (with blue colour) after spectral scanning. COD was measured according to the standard methods [2]. Measured COD and absorbance values were used for the calculation of biodegradation and decolourization efficiencies. The decolourization and COD removal were calculated according to the following formulation Eqs. (1) and (2) [20,21].

$$\text{Decolourization}\,(\%) = \frac{(D_\text{i} - D_t)}{D_\text{i}} \times 100 \quad (1)$$

where D_i is the initial concentration of dye, and D_t the dye concentration along time.

$$\text{COD removal}\,(\%) = \frac{C_\text{i} - C_t}{C_\text{i}} \times 100 \quad (2)$$

where C_i is the initial COD and C_t the COD along the time

The pH was measured using a digital calibrated pH-metre (Inolab, D-82362 Weilheim Germany).

2.4. Factorial design: screening of important factors for colour and COD removal

The effect of three determinant factors (temperature, pH and agitation) known to affect decolourization and COD removal was studied using statistical approach. All these variables were investigated at two widely spaced levels. The complete matrix for screening was designed using the software Minitab v 14.0. A set of eight experiments was carried out to determinate the colour and COD removal under different combinations.

The high and low levels defined for the 2^3 factorial designs are listed in Table 1. The low and high levels for the factors were selected according to some preliminary experiments. The factorial design matrix and measured level-headedness coefficient in each factorial experiment are shown in Table 2, with the low (−1) and the high (+1) levels as specified in Table 1. The level-headedness coefficient was determined as the average of three parallel experiments [24,25].

The order in which the experiments were conducted was randomized to avoid systematic errors. The results were analysed with the Minitab v 14.0 software, and the main effects and interactions between factors were determined.

2.5. Design of the experiment and methods

The D-optional method in the experimental design, provided by the software Minitab (Ver. 14.0, US Federal Government Common wealth of Pennsylvania, USA), was used to optimize the formulation of the above microbial consortium strains. The mixture design was used to study the relationships between the proportion of different variables (*S. paucimobilis*, *Bacillus* sp. and *S. epidermidis*) and responses (colour and COD removal). Ever since, Scheffe devised a single-lattice and single-core design in 1958, the mixture design has developed a variety of methods [26].

RSM is usually applied following a screening study to explore the region of interest of the factors identified by the preceding study. The mixture design is widely used in the formulation of food experiment, chemicals, fertilizer, pesticides and other products. It can estimate the relationship between formulation and performance through regression analyses in fewer experiment times [26].

In this study, *S. paucimobilis*, *Bacillus* sp. and *S. epidermidis* were used as mixture starters, ranging from 0 to 100%, as shown in Table 2. Decolourization and biodegradation experiments were taken according to the ratio given by the experimental design, and 10% v/v of the mixed culture were inoculated into 100 mL of textile wastewater at 28°C for 10 h with shaking (150 rpm) and pH = 7.2. Colour and COD removal were determined after biodegradation.

2.6. Statistical analyses

The statistical analyses were performed by the use of multiple regressions and Analyses of variance (ANOVA) with the software Minitab v 14.0 and Essential Regression v 2.2. The ANOVA analyses were performed between the experimental and theoretical values and the means of the significantly different main effects were compared at $p < 0.05$ [27].

Table 1
Factorial experimental design and observed responses for colour and COD removal (%)

Experiment	Factor			Interaction between the factors				Response					
Low level: −1; High level:+1	Factor: 1; Temperature 28, 37°C	Factor: 2; Agitation 100, 150 rpm	Factor: 3; pH 7.2, 8.5	1.2	1.3	2.3	1.2.3	COD removal %			Colour removal %		
								Experimental	Theoretical	*p* value	Experimental	Theoretical	*p* value
1	−	−	−	+	+	+	−	65.2	69.38	0.685	81.3	81.4	0.99
2	+	−	−	−	−	+	+	44.8	55.25	0.051	51.7	69.51	0.01
3	−	+	−	−	+	−	+	70.9	65.03	0.451	84.8	72.75	0.05
4	+	+	−	+	−	−	−	59.0	50.9	0.144	67.4	60.96	0.36
5	−	−	+	+	−	−	+	42.4	47.54	0.434	47.6	57.12	0.092
6	+	−	+	−	+	−	−	52.6	33.41	0	73.3	45.33	0
7	−	+	+	−	−	+	−	46.1	43.19	0.745	46.7	48.57	0.897
8	+	+	+	+	+	+	+	11.6	29.06	0	20.9	36.78	0.00034
Level-headedness coefficient for COD removal (%)	−7.08	−2.17	−10.9	−4.52	1	−7.15	−6.65						
Level-headedness coefficient for colour removal (%)	−5.89	−4.26	−12	−4.91	5.86	−9.06	−7.96						

The ANOVA analyses were performed between the experimental and theoretical values and the means of the significantly different main effects were compared at $p < 0.05$.

Table 2
Effect of the mixture proportions between the strains on the colour and COD removal (%)

Assay	*S. paucimobilis*	*Bacillus* sp.	*S. epidermidis*	Total	Colour removal (%)				COD removal (%)			
					Experimental	Theoretical	*p* value between experimental and theoretical values	*p* value between the developed consortium and all assays	Experimental	Theoretical	*p* value between experimental and theoretical values	*p* value between the developed consortium and all assays
1	1	0	0	1	90.23	72.75	0.018	0.33	72.52	65.03	0.274	0.92
2	0	1	0	1	52.57	72.75	0.0022	0*	75.76	65.03	0.075	0.96
3	0	0	1	1	67.07	72.75	0.51	0*	70.98	65.03	0.441	0.77
4	0.50	0.5	0	1	80.20	72.75	0.318	0.05*	68.37	65.03	0.773	0.46
5	0.50	0	0.50	1	90.56	72.75	0.0144	0.36	67.82	65.03	0.835	0.40
6	0	0.50	0.50	1	83.41	72.75	0.096	0.02*	75.76	65.03	0.07252	0.96
7	0.33	0.33	0.33	1	98.67	72.75	0	–	74.52	65.03	0.125	–
8	0.66	0.16	0.16	1	85.53	72.75	0.034	0.07	67.52	65.03	0.866	0. 37
9	0.16	0.66	0.16	1	80.50	72.75	0.289	0.006*	71.88	65.03	0.338	0.86
10	0.16	0.16	0.66	1	99.93	72.75	0.00001	0.97	64.31	65.03	0.988	0.122

The ANOVA analyses were performed between the experimental and theoretical values and the means of the significantly different main effects were compared at $p < 0.05$.
*$p < 0.05$: (significant difference) the experimental values of the developed consortium (assay 7) and all combinations was determined for colour and COD removal

3. Results and discussion

3.1. Interaction and contour plots for colour and COD removal

The interaction plots for colour and COD for all variables is shown in Figs. 1 and 2, respectively. The agitation showed a positive effect on the colour and COD removal (Figs. 1(d) and 2(d)) using pH = 7.2 and $T = 28$ °C to reach the optimum with the agitation of 150 rpm. For the interaction between temperature and pH (Figs. 1(b) and 2(b)), we concluded that to slightly alkaline pH, temperature had no effect on fading. The pH showed a negative effect on the fading, which is more remarkable by increasing agitation at 150 rpm and at a temperature $T = 37$ °C (Figs. 1(d) and 2(d)).

Higher colour removal at different pH showed that the maximum value was obtained at 7.2 compared to pH = 8.5 as shown in Figs. 1 and 2. The regression model can give an idea of fading if we change the factors [28]. The necessary condition which is not sufficient to have a maximum rate of COD removal is pH close to the neutrality, thus following the contours of curve corresponding to a fixed pH of 7.2 (Fig. 3(b)) can detect the optimal conditions of the COD removal which are: a pH of 7.2, a temperature of 28 to 29 °C and agitation ranging from 145 to 150 rpm. The contours curve (Fig. 3(a) and 3(b)) generated by the software MINITAB 14.0 [29] and corresponding to the manipulation at a fixed temperature of 28 °C offers more opportunities to the work at rates of significant fading. So, we can draw here the optimal conditions for the decolourization and biodegradation which are: a temperature of 28 °C, a pH close to the neutrality (7.2–7.25) and a restlessness of 150 rpm. The present results showed essentially no deactivation of biological activity under operational temperatures. Therefore, the used organisms could acclimatize to broad range of temperatures.

The contour plots for colour and COD removal as shown in Fig. 3(a) and 3(b), respectively, indicated the efficiency of colour removal as a function of various variables. The maximum colour removal of above 84.8% was obtained with the agitation of 150 rpm, a pH which ranged between 7.2 and 7.25 and with a temperature of 28 °C. Maximum COD removal of above 80% was obtained at the agitation ranging between 145–150 rpm, with a pH of 7.2 at a temperature of 28–29 °C. These results significantly ruled out any further significant rise in colour and COD removal by increasing the concentration.

The optimal temperature for bacterial activity was determined by research groups as ranging between 27–35 °C. However, further increase in temperature beyond that resulted in marginal reduction in dye decolourization but essentially thermal deactivation of decolourization activity under operational temperatures did not occur. Decline in bacterial activity at higher temperatures (>45 °C) may be attributed to loss

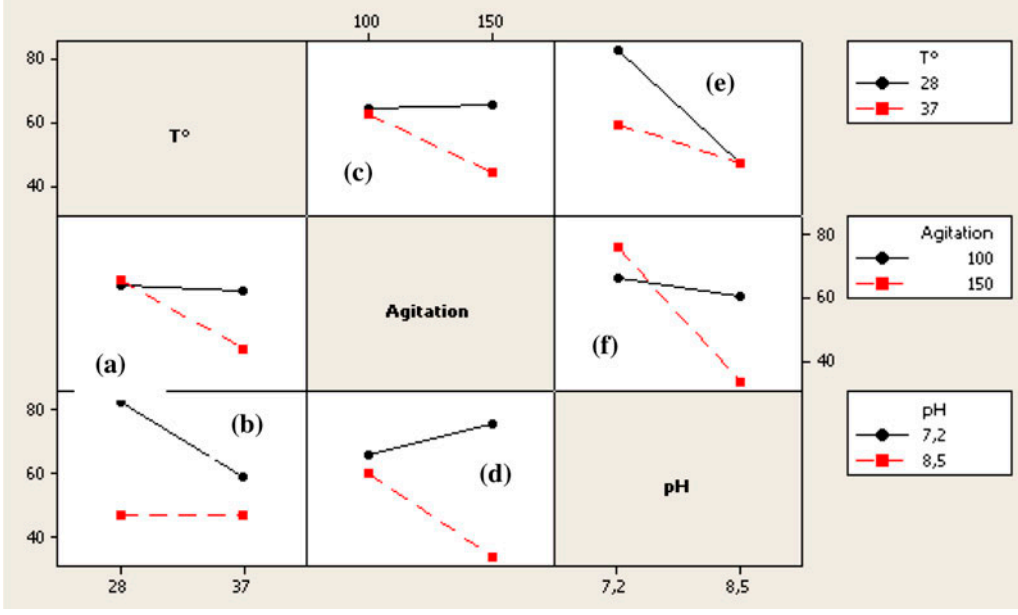

Fig. 1. Interaction plot for % colour removal: (a) interaction between T°/agitation; (b) interaction between T°/pH; (c) interaction between agitation/T°; (d) interaction between agitation/pH; (e) interaction between pH/T°; and (f) interaction between pH/agitation.

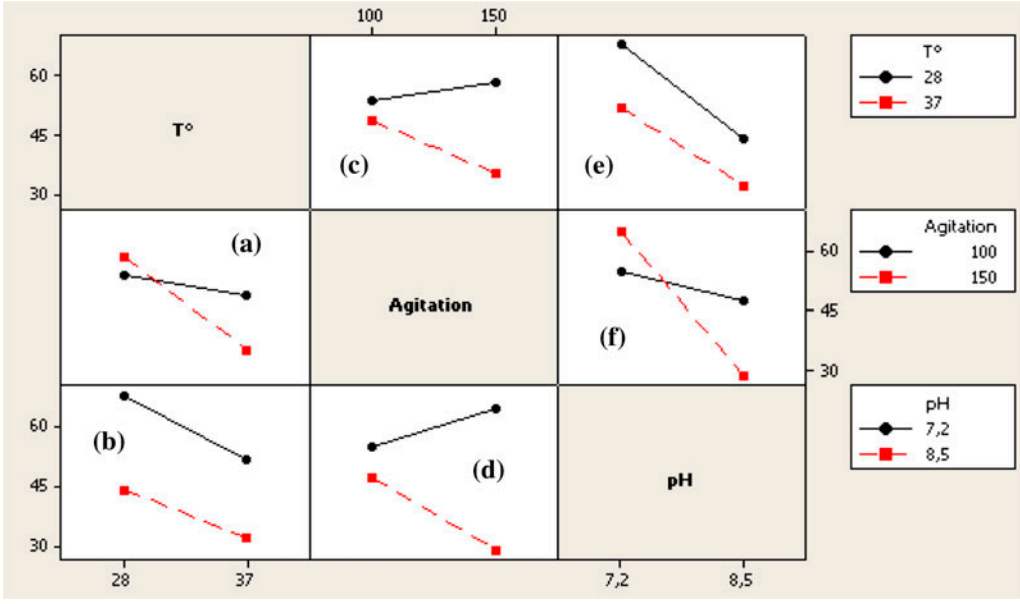

Fig. 2. Interaction plot for COD removal (%): (a) interaction between T°/agitation; (b) interaction between T°/pH; (c) interaction between agitation/T°; (d) interaction between agitation/pH; (e) interaction between pH/T°; and (f) interaction between pH/agitation.

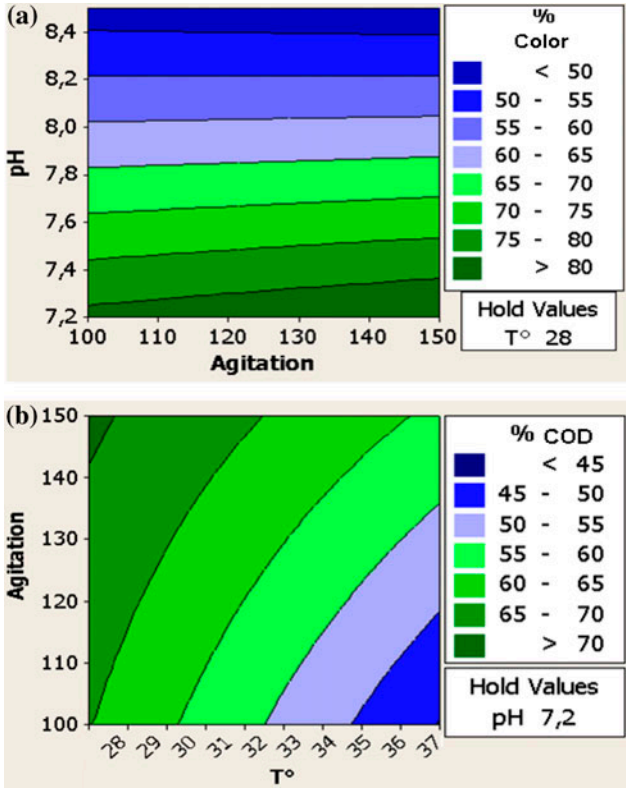

Fig. 3. Contour plots for color (a) and COD (b) removal.

of cell viability or denaturation of the catabolic enzyme. Determination of temperature requirements of micro-organisms used for biotechnological applications is paramount, since temperature requirements above ambient ranges may require an energy input and hence are not cost-effective [30]. The incubation under agitated condition is also necessary for better cell growth, in contrast to incubation under static condition. Poor decolourization of the dyes obtained under a static condition could be attributed to the limitation of oxygen needed for the oxidative breakdown. Tolerance to varying pH by dye-decolourizing bacteria is quite significant, as it makes them suitable for practical biotreatment of dyeing mill effluents. However, to achieve the best rate of degradation, it is suggested that the pH of textile effluent should be neutralized to 7. This tendency of decolourization dependence on pH has been reported by many researcher groups [31]. They have found that pH between 7 and 9 is optimum for the decolourization of dyes. The effect of pH may be related to the transport of dye molecules across the cell membrane, which is considered a rate-limiting step for dye decolourization. At pH below 4, H^{+} ions compete effectively with dye cations causing a decrease in colour removal efficiency. But at higher pH above this point charge, the surface of biomass gets negatively charged, which attracts the positively charged dye cations through electrostatic force of attraction [30].

3.2. ANOVA test

The statistical significance of the ratio of mean square due to regression and mean square residual error was tested. The "*P*" values obtained were lower which indicated that the factors played a significant role and that the model was statistically significant [32]. The regression coefficients "*F*" and "*p*" values for the analyses are given in the Table 3. The regression models for efficiency of colour and COD removal are given in following equations:

$$Y_{\%\ \text{decolourization}} = 269 - 1.31\ T^{\circ} - 0.171\ \text{Agitation} - 18,6\ \text{pH}$$

$$Y_{\%\ \text{COD}} = 243 - 1.57\ T^{\circ} - 0.087\ \text{Agitation} - 16,8\ \text{pH}$$

3.3. Effect of formulation on the colour and COD removal

In the mixture design, the effect of variables (*S. paucimobilis*, *Bacillus* sp. and *S. epidermidis*) changing on the responses (colour and COD removal) can be observed on the ternary contour map. In this study, three variables were studied. In order to confirm the experimental results that 100 and 75.76% were obtained, respectively, for colour and COD removal, a mixture contour plot was plotted by MINITAB® 14 Software Programme. The mixture contour plots between the variables *S. paucimobilis*, *Bacillus* and *S. epidermidis* are given in Fig. 4. The lines of the contour plots predicted values of each response at different proportions of *S. paucimobilis*, *Bacillus* sp. and *S. epidermidis*. When the strains were combined, the decolourization and the biodegradation yields increased. In fact, these yields, as presented in Table 2, increased (assay number 7) with yields of colour and COD removal efficiencies of 98.67 and 74.52%, respectively, compared with the single strains (assay number 1, 2 and 3). So, this combination between these strains may be efficient as it enhances the decolourization that is attributed to both degradation and absorption of the dye on the surface cell. According to Axelord and Hamilton [33], if two bacteria have the continuous contact (e.g. in closed systems like batch reactors or shake-flask cultures) and each organism can benefit from mutual cooperation, then each one can also do even better by exploiting the cooperative efforts of others. Yet, for some bacteria, their presence still extensively enhances the decolourization activities of other strains due to extracellular metabolites released as stimulators/enhancers. In fact, the enzymatic activity of a single strain is highly influenced by the presence of other micro-organisms, and the biocatalytic activity of a consortium is different from that of its individual constituents. Micro-organisms in a consortium work synergistically to enhance decolourization activity [34].

Dye decolourization using pure culture and/or by consortium has been reported by various researchers. The obtained results in this work are in agreement with other works which showed that individual strains could not completely degrade textile wastewater [35,36] such as *Streptomyces* spp. [37], *Phanerochaete chrysosporium* and *Pseudomonas luteola* [38]. However, single microbial strains are able to decolourize dyes, but degradation products are frequently toxic aromatic amines or metabolites that are more difficult to biodegrade than the parent dye. Furthermore, these micro-organisms are often specific to one type of dye; and due to the chemical complexity of wastewater from the textile industry, several groups have attempted to develop more efficient microbial processes. The complete degradation of chemical substances is possible with the aid of several enzymatic reactions, which implies the necessity of building microbial consortia [39,40]. A significant advantage of consortia over the use of single strains in the degradation of dyes is that different strains can attack the colorant molecule at different positions or can use the metabolites produced by another strain for further decomposition, in some cases attaining the mineralization of dyes. Thus, the utilization of microbial consortia offers a considerable advantage over the use of pure cultures. As the catabolic activities of microorganisms in the consortium complement each other, syntrophic interactions present in the mixed communities can lead to complete mineralization of dyes [41].

The developed consortium may be effective for the treatment of textile wastewaters considering the results of a total colour removal, (100%) and a 75.76% COD removal obtained compared with other research groups. In fact, Nigam et al. [24] obtained the yield of 80% of colour removal by using bacterial cultures, treating mixture of azo and diazo reactive dyes, and the yields obtained by Banat et al. [25] treating textile dyes by an isolated bacterial culture with a colour removal ranging between 67 and 84%. Patil et al. [42] developed the consortium PMB11, which consisted of three bacterial species, *Bacillus odysseyi* SUK3, *Morganella morganii* SUK5 and *Proteus* sp. SUK7, thus completely decolourizing Reactive Blue 59 (50 ppm) in 3 h, while the individual bacterial strains decolourized this dye in more than 24 h [42]; the same consortium decolourized 99% of Red HE3B (50 ppm) within 12 h in nutrient broth, whereas the individual strains decolourized between 85 and 89% of Red HE3B in more than 60 h [43]. Phugare et al. [39] developed the consortium SDS, which consisted of *Providencia* sp. and *Pseudomonas aeruginosa*, completely decolourizing

Table 3
ANOVA for colour and COD removal

	Source	Degrees of freedom	Sum of square	Sum of adjusted squares	*F*-ratio	*p*-value (significance)
ANOVA for colour removal (%)	Regression	3	1591.5	530.5	1.30	0.039
	Residual error	4	1632.3	408.1	–	–
	Total	7	3223.8	–	–	–
ANOVA for COD removal (%)	Regression	3	1388.8	462.9	1.98	0.025
	Residual error	4	934.6	233.6	–	–
	Total	7	2323.3	–	–	–

Red HE3B and Remazol Black 5B and 97% of Red HE7B (50 ppm) in 1 h, but the single strains take between 5 and 48 h [39]. *P. aeruginosa* causes up to 92% decolourization of a textile effluent within 30 h, and *Providencia* sp. achieves 84% decolouration within 48 h, while the consortium of both micro-organisms is responsible for complete effluent decolourization in 20 h [44]. The decolourization of six reactive dyes using a consortium of *P. vulgaris* and *Micrococcus glutamicus* is significantly higher than with individual micro-organisms [45]. It is anticipated that all species in the microbial community may make use of their metabolic activities to reach a goal of pollutant degradation or detoxification for total survival. This microbial community should be well balanced. This balanced distribution of the ecosystem leads to improving the colour and COD removal of the treated wastewater [12]. However, in the construction of consortia, the proportion of each micro-organism is important to obtain an efficient system for the treatment of dyes. For example, an equal proportion of *Bacillus cereus*, *P. putida*, *Pseudomonas fluorescens* and *Stenotrophomonas acidaminiphila* isolated from contaminated locations demonstrate a threefold increase in decolourization efficiency compared with other combinations of the same cultures [46]. The treatment systems having mixed microbial populations are more effective due to concerted metabolic activities of microbial community [16,47,48]. In fact, significant advances have been made in the microbiological and biochemical areas. These advances have been driven by the development of new analytical techniques that allow micro-organisms to be studied *in situ* [49].

The ANOVA analyses were performed, and the means of the significantly different main effects were compared at $p < 0.05$. For all results (Table 2), we calculated the p value between the experimental and theoretical results using ANOVA analyses.

When the isolates were combined, the colour and COD removal increased with yields of colour and COD removal efficiencies of 98.67% (the differences between the experimental and the theoretical values for colour removal with p value of 0 which is <0.05 indicating a significant difference between these values for colour removal) and 74.52% (the differences between the experimental and the theoretical values for colour removal with p value of $0.125 > 0.05$ indicating no significant difference between these values for COD removal), respectively, compared with the single

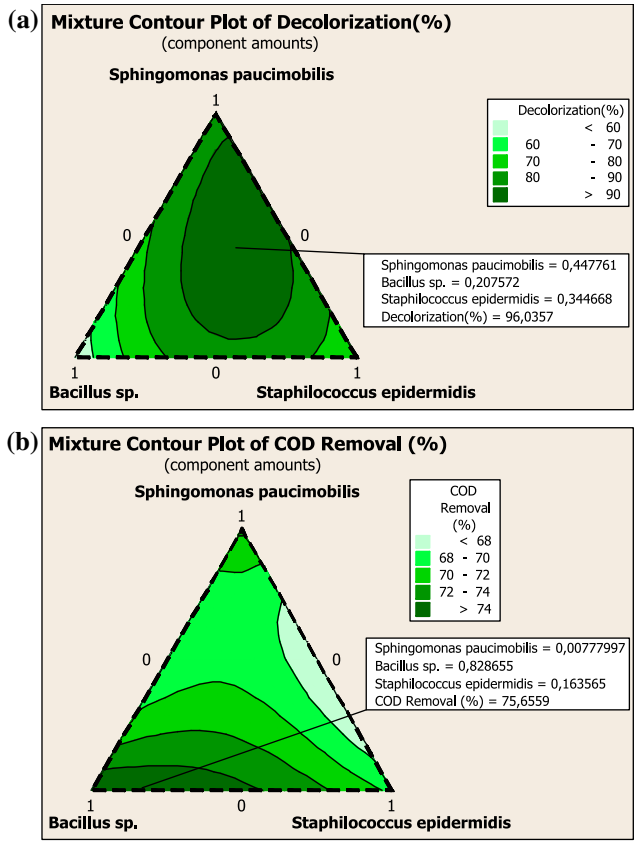

Fig. 4. Mixture contour plots between the variables (*S. paucimobilis*, *Bacillus* sp. and filamentous bacteria contents) for colour and COD removal.

strains with maximal obtained values of single strains of colour and COD removal efficiencies of 90.23% (the differences between the experimental and the theoretical values for colour removal with p value of 0.018 which is <0.05 indicating a significant difference between these values for colour removal) and 72.52% (the differences between the experimental and the theoretical values for colour removal with p value of $0.274 > 0.05$ indicating no significant difference between these values for COD removal), respectively.

The calculated p values between the experimental values of the developed consortium (combination 7) and all combinations were determined for colour and COD removal. No significant difference was observed only for colour removal efficiencies and for the combination 1 of the single strain *S. paucimobilis* with p value of $0.33 > 0.05$ (indicating no significant difference). For the two other combinations 2 and 3 of single strains, the results showed a significant difference with the developed consortium with p values of $0 < 0.05$. We showed that for the developed consortium, an increase of colour and COD removal efficiencies was compared with the single strains. This improvement is not significant, but there is a slightly increase in these two yields.

4. Conclusion

This study clearly shows that RSM is one of the suitable methods to optimize the best operating conditions for target value of colour and COD removal. However, the contours curve generated by the software MINITAB and corresponding to the manipulation at a fixed temperature of 28˚C offers more opportunities to the work at rates of significant fading. We showed that the optimal conditions for the decolourization are a temperature of 28˚C, a pH close to the neutrality (7.2–7.25) and a restlessness that ranges from 100 to 150 rpm. The decolourization and the biodegradation yields slightly increased with the developed consortium compared with single strains, with yields of colour and COD removal efficiencies of 98.67 and 74.52%, respectively. High colour and COD removal efficiencies of this microbial consortium indicate its potential use in antipollution treatments. However, only a better understanding of the mechanisms used will allow applying it to cleaning up aquatic and terrestrial environments.

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