Valorization of the *Vicia faba* mucilage on textile wastewater treatment as a bio-flocculant: process development and optimization using response surface methodology (RSM)

Feriel Bouatay, Nesrine Eljebsi, Sonia Dridi-Dhaouadi and Farouk Mhenni

**ABSTRACT**

The *Vicia faba* membranes are an abundant and a low cost product. In the present research paper, the extracted *Vicia faba* mucilage was tested as an eco-friendly flocculant for textile wastewater treatment. Its performance as flocculant, in decolorization, chemical oxygen demand (COD) removal and the concentration of total suspended solids was checked. The natural extracted product was characterized using infrared spectroscopy. The total sugars were determined in the extracted product. The effect study, followed by an optimization and modeling analysis, of some experimental parameters on the coagulation–flocculation performance, using *Vicia faba* mucilage (as a flocculant), combined with aluminum sulfate (as a coagulant), showed that the best conditions for the flocculation process were pH of the effluent about 7, flocculant dose about 6.75 mg/L, flocculation mixing time about 3 min and flocculation mixing speed about 30 rpm, leading to a decolorization equal to 92.32%, COD removal of about 97.52% and total suspended solids of about 15.3 mg/L. A comparison study between the flocculation performance of commercial reagents and the bio-agent showed that the natural product presented a good flocculation performance.

**Key words** | bio-flocculant, COD removal, decolorization, total suspended solids, *Vicia faba* membranes

**INTRODUCTION**

Dyeing and finishing denim fabric industries are the world’s fastest growing sector due to ever increasing demand for its products. These industries are one of the biggest users of water and chemical products. Their effluent contains residual chemical products in water and presents a high level of suspended solids and organic materials. So, this wastewater is high in color, chemical oxygen demand (COD), biochemical oxygen demand, pH, temperature, turbidity and toxic chemicals. The direct discharge of this effluent into the nature affects the flora and fauna and pollutes water. Therefore, wastewater treatment is indispensable for these wastewater industries. So, several effluent treatment methods have been developed during the past years. These methods include filtration (Saffaj et al. 2005), cation exchange membranes (Wu et al. 2008), electrochemical degradation (Fan et al. 2008), photo-Fenton (Núñez et al. 2007), biological treatment (Lu et al. 2009; Wang et al. 2009), adsorption (Errais et al. 2012; Bouatay et al. 2014), oxidation (Muneer et al. 2015; Saeed et al. 2015, 2016) and coagulation–flocculation (Gao et al. 2007; Kumar et al. 2008). In this regard, this last is one of the most used technologies for textile industry effluent treatment. In fact, this wastewater is usually milky with dyes and chemical products, containing dispersed solid particles called colloids which are stabilized by negative electric charges on their surface, causing the repulsion between these particles and keeping them in suspension. So, it requires the addition of chemicals to neutralize the surface colloidal particle charges (Sher et al. 2015). Therefore, the pollution removal mechanism of this process is mainly due to the neutralization of the negative colloid charges using cationic hydrolysis products, which allows the van der Waals force of attraction to encourage initial aggregation of colloidal particles to microflocs (Ebeling 2003). Furthermore, the effectiveness of coagulation...
treatment depends on the coagulation agent used, the dose, the wastewater pH, the concentration and the nature of organic materials in the effluent. In this regard, aluminum sulfate was the most used coagulant in textile wastewater treatment due to its low cost, storage and performance. Following the first process of coagulation, a second step called flocculation occurs. This increases the particle size from sub-microscopic microfloc to visible suspended solids. In fact, in this step, a high molecular weight polymer, called coagulant aid or flocculant, is added to help collisions of microflocs, bridge, bind and particle strengthen the floc, add weight and then increase settling rate. Moreover, considerable research has been focused on the removal of pollution from textile effluent treatment by the coagulation–flocculation process using conventional and synthetic reagents as coagulants and flocculants (Gao et al. 2007; Sher et al. 2013). Recently, researchers concentrated their studies on using low cost, abundant, biodegradable and non-conventional products to reduce the toxicity of the wastewater and the cost of treatment. In this regard, several studies in recent years have focused on the use of various low cost, abundant and non-conventional bio-flocculant to reduce the toxicity of the effluent and the treatment cost. Khiari et al. (2013) evaluated the use of sodium carboxymethylcellulose (CMCNa) prepared from date palm rachis as an eco-friendly flocculant. In addition, plant-derived polysaccharides are considered as an environmentally friendly flocculant due to their biodegradability, stability and low cost. In this regard, Mishra & Bajpai (2005) studied the application of a food grade polysaccharide, namely Plantago psyllium mucilage, for the removal of vat and reactive dyes. Mishra & Bajpai (2006) studied the flocculation performance of Tamarindus mucilage in the removal of vat and direct dyes. Also, Anastasakis et al. (2009) assessed the flocculation behavior of mallow and okra mucilage in wastewater treatment. Bouatay & Mhenni (2014) studied the flocculation performance of cactus mucilage in an industrial textile wastewater. The present research paper aims to valorize the Vicia faba membranes mucilage as an eco-friendly flocculant. This plant is cheap and easily available. It contains 22.4–36% protein, 57.8–61% carbohydrates, 12% fiber and 1.2–4% lipids (Hedley 2003). In this research paper, the use of Vicia faba membranes mucilage as a natural flocculant for an industrial wastewater taken from the dyeing and finishing unit of a denim fabrics industry was investigated. The effects of the main experimental conditions (pH of the effluent, flocculant dose, flocculation mixing speed and flocculation mixing time) on the flocculation treatment performance were studied. Also, modeling and optimization of some experimental conditions were performed in order to improve the performances of the flocculation process. Comparison with commercialized anionic and cationic flocculants was made under the optimal conditions. The flocculation process performance in all cases was evaluated by means of the decolorization, the COD removal and total suspended solids (TSS) concentration.

**MATERIALS AND METHODS**

**Water characteristics**

Laboratory tests were conducted on real effluent taken from a Tunisian dyeing and finishing unit for denim fabrics and conserved at temperature of 5 °C.

The UV-visible (UV/Vis) spectrum of the industrial wastewater (diluted 10 times) was recorded using a Cecil Instruments 2021 UV/Vis spectrophotometer and the result is represented in Figure 1. The studied effluent characteristics are given in Table 1.

![UV/Vis spectra of different studied dye baths.](https://iwaponline.com/wst/article-pdf/75/3/629/455703/wst075030629.pdf)

**Table 1** Studied effluent characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>11.9</td>
</tr>
<tr>
<td>Maximum wavelength</td>
<td>650 nm</td>
</tr>
<tr>
<td>Absorbance</td>
<td>10.67</td>
</tr>
<tr>
<td>COD (mg O₂/L)</td>
<td>2,350</td>
</tr>
<tr>
<td>Conductivity</td>
<td>3.6</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>38</td>
</tr>
</tbody>
</table>
Flocculant characteristics

In the chemical water treatment, the flocculants used were EPENWATE EXP 31/1 (Pentex Quimica, Spain), polyacrylamide A\textsubscript{100}PWG (Kemira Chemicals, France) and \textit{Vicia faba} mucilage. Their characteristics are presented in Table 2.

Mucilage extraction

The \textit{Vicia faba} membranes were naturally collected in March 2014 from the eastern Tunisian region. They were repeatedly washed with distilled water to remove dirt particles, sun dried for 2 h and cut into small pieces. The plant was then powdered using a domestic mixer to obtain a viscous juice. To obtain the mucilage, about 800 g of the juice were soaked in 1 L of distilled water and stirred at 300 rpm for 12 h at 25 \textdegree C. The separation of mucilage and fibers was done by filtration of the solution through a muslin cloth. The mucilaginous extract was then precipitated by adding three parts of ethanol (85\%) to one part of the solution and kept at 4 \textdegree C for 2 h. In order to obtain the pure extract, the solution was centrifuged at 3,000 rpm for 15 min. Finally, the obtained bio-agent was stored in a glass bottle for further use as flocculation agent.

FTIR analysis

A Fourier transform infrared (FTIR) spectrum of the bio-flocculant was recorded using a Shimadzu 8400 FTIR spectrometer (Japan), with the processing software Hyper 1.57 using bromide disks. A total of 32 scans for the sample were taken with a resolution of 4 cm\(^{-1}\), with a range of 4,000–400 cm\(^{-1}\).

Determination of neutral sugars

The content of sugars in the \textit{Vicia faba} extract mucilage was determined by using the phenol-sulfuric acid method, as described by Dubois \textit{et al.} (1956). Sulfuric acid (analytical grade 95\%, specific gravity 1.84) used was purchased from Aldrich, France.

For the blank solution, 1 mL of distilled water was added to 1 mL of 5\% phenol followed by 5 mL of concentrated sulfuric acid. For the standard solution, a 100 \mu g/mL stock solution of glucose was prepared in distilled water. Then, aliquots were taken from this solution to obtain sugar concentrations of 60–90 \mu g/mL. To 1 mL of 5\% phenol solution was added 1 mL of sugar solution prepared previously, followed by 5 mL of concentrated sulfuric acid. The absorbance of the obtained solutions was measured after 10 min at 480 nm against a blank using a Cecil Instruments 2021 UV/Vis spectrophotometer. To estimate the neutral sugars content in the \textit{Vicia faba} mucilage, 1 mL of the 5\% phenol was added to 1 mL of the \textit{Vicia faba} extract followed by 5 mL of concentrated sulfuric acid. The absorbance was measured after 10 min at 480 nm. The following experiments were carried out in triplicate.

Determination of uronic acid

The uronic acid in the \textit{Vicia faba} mucilage was determined by using the carbazole method described by Disch (1974). To 0.5 mL of the sample, 3 mL of 0.025 M sodium tetraborate prepared in concentrated sulfuric acid was added. The tubes were refrigerated in crushed ice. The mixture was shaken in a vortex mixer and the tubes heated in a water bath at 100 \textdegree C for 10 min. After cooling in a water-ice bath, 0.2 mL of carbazole 0.1\% (w/v) made in absolute ethanol was added. The obtained solution was shaken and heated to 50 \textdegree C for 30 min, then to 100 \textdegree C for 30 min. After refrigeration, the absorbance of the

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Flocculant characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Appearance</strong></td>
<td>EPENWATE EXP 31/1</td>
</tr>
<tr>
<td>Chemical characteristics</td>
<td>Powdery, white, granular solid, odorless</td>
</tr>
<tr>
<td>Water-soluble cationic polymer, positive charges present on chain, polyelectrolyte</td>
<td>Water soluble anionic polymer, negative charges present on chain, polyelectrolyte</td>
</tr>
<tr>
<td>Density</td>
<td>High</td>
</tr>
<tr>
<td>Molecular weight (g/mol)</td>
<td>(2.5\times10^5) to (10^6)</td>
</tr>
</tbody>
</table>

The EPENWATE Exp31/1 and polyacrylamide A\textsubscript{100}PWG are, respectively, commercial cationic and anionic flocculants used in wastewater treatment.
solution was measured at 530 nm using a Cecil Instruments 2021 UV/Vis spectrophotometer. For a standard curve, 0 to 100 μg/mL of galacturonic acid was used. The following experiments were carried out in triplicate.

**Jar tests**

The flocculation treatment was combined with coagulation treatment using aluminum sulfate, Al₂(SO₄)₃ (Aldrich, France). To evaluate the flocculation performance of the *Vicia faba* mucilage, jar tests were carried out with special flocculating equipment (Flocculateur W10408, Fisher Bioblock, Germany). The flocculating device was equipped with four beakers to be mixed simultaneously with the same stirring speed (0–500 rpm). The jar tests were batch experiments occurring in three steps: the first one was a rapid mixing coagulation (200 rpm for 5 min) in which 3 mL of a coagulant solution (1 g/L of aluminum sulfate) was added to 100 mL of untreated dyeing waste solution; the second step was a slow mixing flocculation treatment (20–100 rpm for 2–20 min) in which a fixed quantity of the bio-agent obtained previously (2–10 mg) was added to 100 mL of the effluent used after coagulation; and the third step was a separation of solid and liquid in which the solution was left for sedimentation for half an hour.

After sedimentation, the supernatant was analyzed in terms of decolorization, COD abatement, turbidity removal and TSS content. The following experiments were carried out in triplicate.

The absorbance of the supernatant was recorded using a Cecil Instruments 2021 UV/Vis spectrophotometer. The color removal (decolorization (%)) was calculated according to Equation (1):

\[
\text{Decolorization (\%)} = \left(\frac{\text{Abs}_i - \text{Abs}_f}{\text{Abs}_i}\right) \times 100 \tag{1}
\]

where \(\text{Abs}_i\) and \(\text{Abs}_f\) are the absorbance (measured at the maximum wavelength) of the dye bath solution and the supernatant after coagulation–flocculation treatment, respectively.

The COD removal was measured according to the standard method and expressed as CODcr (potassium dichromate as oxidant) (APHA 1999).

The COD removal (%) was calculated according to Equation (2):

\[
\text{COD removal (\%)} = \left(\frac{\text{COD}_i - \text{COD}_f}{\text{COD}_i}\right) \times 100 \tag{2}
\]

where \(\text{COD}_i\) (mgO₂/L) and \(\text{COD}_f\) (mgO₂/L) are the COD of the effluent before and after coagulation–flocculation treatment, respectively.

The turbidity was determined by a Turb 555 IR turbidity meter according to the APHA (1999) standard method.

The turbidity abatement (Tur) was calculated according to Equation (3):

\[
\text{Tur (\%)} = \frac{\text{Tur}_i - \text{Tur}_f}{\text{Tur}_i} \times 100 \tag{3}
\]

where \(\text{Tur}_i\) (NTU) and \(\text{Tur}_f\) (NTU) are the turbidity values of the dye bath solution before and after coagulation–flocculation treatment, respectively.

The TSS was measured according to standard method APHA 2540 (APHA 1999).

**Design of experiment**

Effect studies of the different factors (pH, flocculant dose, mixing speed, mixing time) and optimization of the flocculation conditions were investigated using response surface methodology (RSM). This is an efficient statistical approach to represent the effect and the interaction between the different studied variables and for optimizing multifaceted processes. RSM minimizes the number of experimental trials required to evaluate several parameters and their interactions.

Regression analysis and analysis of variance (ANOVA) were used to study the effect of each parameter selected on the obtained results; Minitab (Version 15, State College, PA, USA) was used for the statistical analysis of data. Comparison of means was conducted using ANOVA with post hoc Tukey’s test at \(P < 0.05\) (Carmona et al. 2005).

**RESULTS AND DISCUSSION**

**Characterization of the *Vicia faba* mucilage**

The FTIR spectrum was used to identify the presence of functional groups on the extracted dye. Figure 2 shows the FTIR spectrum of the *Vicia faba* extracted dye. For *Vicia faba*, a broad absorption peak at around 3,200–3,500 cm⁻¹ is observed, indicating the presence of carboxylic acid. The absorption band at 2,993 cm⁻¹ could be assigned to asymmetric vibration of CH. The stretching vibration band at 1,614 cm⁻¹ is due to asymmetric stretching of the carboxylic C=O double bond. A 1,428 cm⁻¹ is the peak of phenolic
-OH and –C=O stretching of carboxylates. A 1,231 cm⁻¹ band is the stretching vibration of –COO. The band at 1,016 cm⁻¹ could be due to the vibration of –C–O–C– and –OH of polysaccharides (Barka et al. 2015). Furthermore, the presence of hydroxyl groups favored the possibility of hydrogen bonding with dye molecules and increased the adsorption of these molecules onto the bio-flocculant surface. So, the molecules of dyes presented in the effluent exhibited high solubility. In fact, OH, COOH or COO⁻ group of the bio-flocculant and H⁺ or OH⁻ group on the surface of particles might form hydrogen bonds as the bio-flocculant chains approach the surface of molecule dyes. The bridging mechanism occurs after the particles have adsorbed onto the bio-flocculant surface. Many pollutant particles could adsorb to a long molecular chain, and the particles adsorbed onto the bio-agent could be adsorbed simultaneously by other bio-flocculant chains, leading to the formation of three-dimensional flocs capable of settling rapidly (Deng et al. 2005). Thus, the bio-flocculant has good flocculating performance.

In this regard, the determination of the total sugars concentration showed that the total sugars in the extracted Vicia faba mucilage was about 37.25%. Moreover, the results related to the amount of the uronic acid showed that the obtained bio-agent presented a high percentage of this acid sugar of about 19.11%. So, this indicated that the mucilage extract presented an important amount of pectic polymers and polysaccharide (Habibi et al. 2004). This polymer presented a high amount of carboxyl groups which provided more effective sites for pollutants attachment and many particle can be adsorbed onto the bio-flocculant chain.

On the other hand, natural polymers, mainly polysaccharides, are becoming used as a bio-flocculant in water treatment. In fact, they are biodegradable, easily available from reproducible resources and usually non-toxic (Okuda et al. 1999). So, in this research paper, the flocculation performance of the obtained agent was investigated.

Effect of the flocculation conditions on the treatment efficiency

The coagulation–flocculation process was carried out in four steps. In the first step, the pH of effluent was adjusted to 7 as the optimum pH for aluminum sulfate (Gebbie 2009). In the second step, the colloidal particle was destabilized after the addition of coagulant (aluminum sulfate). In the third step, pH was adjusted with dilute sulfuric acid or sodium hydroxide. In the final step, flocculant was added to bridge the destabilized colloidal particles together to develop larger flocs. This same procedure was repeated for all the experiments in which the pH, the flocculant dose, the mixing speed and time were varied. The effect of these parameters on the COD removal,
decolorization and suspended solids was analyzed. The obtained results are given in Figure 3.

**Effect of the effluent pH**

Figure 3(a) shows the removal of pollution as a function of pH for real effluent taken from the textile industry. It is obvious that the pH changes do not have an important effect on the COD removal. On the other hand, the maximum decolorization was observed at neutral pH. Also, the minimum TSS was seen at about pH 7 and 8. So, the pH changes do not affect the efficiency of natural polymers. Therefore, the decrease and increase observed in decolorization was due to changes in the dye structures and their conversion from solubilized form into non-solubilized form. So, the best flocculation performance was achieved when the pH was above 7. The removal efficiency of the color and COD were, respectively, 91.2% and 96.7% at neutral pH. In fact, the maximum removal of pollution at neutral pH might be attributed to the hydrogen bonding between the functional groups of polymer and the dye (Bekturov et al. 1997).

**Effect of the flocculant dose**

The plots in Figure 3(b) show the decolorization, COD removal and the TSS of the treated effluent versus the flocculant dose. It was found that with an increase in flocculant dose up to certain level, the flocculation performance increased, which was followed by a decreasing trend in dye removal with further increases in dose level.

The most effective dose of the flocculant was found to be 5 mg/L at which the best flocculation performance was observed.

This trend (increasing and then decreasing trend) in decolorization and COD abatement was because of the fact that the optimum amount of mucilage in the suspension caused larger amounts of pollutant particles to aggregate and settle. However, an excess amount of the flocculant dose above the optimal in effluent would cause the aggregated particle to respread and would also disturb particle settling (Chan & Chiang 1995). This behavior could be explained by the repulsive energy between the polymer and the pollutant after the increasing of the flocculant dose, which causes hindrance in floc formation (Mishra & Bajpai 2006).

![Figure 3](https://iwaponline.com/wst/article-pdf/75/3/629/455703/wst075030629.pdf)

**Figure 3** Effect of the flocculation experimental conditions on the decolorization, COD removal and TSS: (a) effect of the effluent pH, (b) effect of the flocculant dose, (c) effect of the mixing speed, (d) effect of the mixing time.
Effect of flocculation mixing speed

Figure 3(c) shows the removal of pollution as a function of the flocculation mixing speed. From these plots, it is observed that with an increase in mixing speed up to a certain level, the flocculation performance increased, followed by a decreasing trend in dye removal with further increases in speed level. Figure 3(c) shows a transition value around 50 rpm. For a lower mixing speed, the decolorization and COD removal were relatively high and attained, respectively, about 89.7% and 95.9%. However, up to 50 rpm, the flocculation treatment performance undergoes a notable decrease to reach 87.3% for the decolorization and 90.4% for the COD removal. Furthermore, the TSS increases from 4.4 mg/L for 30 rpm to 18 mg/L for 120 rpm. In fact, the higher mixing speed leads to the breakage of the aggregated particles (Khiari et al. 2010).

Effect of flocculation mixing time

The effect of the flocculation mixing time on the flocculation treatment performance is shown in Figure 3(d). The maximum removal of the pollution was found to occur at about 3 min. When the mixing time was higher than 3 min, the decolorization, the COD removal and the decolorization were constant. This showed that the maximum performance of the flocculation process was attained for 3 min of mixing time. Figure 3(d) shows that the amount of decolorization and COD removal increased up to the optimal mixing time. Furthermore, the TSS increases for a mixing time higher than 3 min. So, Figure 3(d) shows three distinct phases: the first one represented the interaction of the pollutant with flocculant, which caused destabilization of the particles in suspensions and they began to flocculate. The second phase indicated the slight decrease of the pollutant removal due to destabilization of the aggregated particles (Agarwal et al. 2005). The third phase showed attainment of the stability by the flocs.

Modelling and optimization of the flocculation process

In this research paper, the process optimization is achieved by the study of a response surface plan. The RSM consists of an empirical modeling technique, which has been used to evaluate the relationship between the experimental and the predicted results (Rozic et al. 2010). Using an experimental design, we investigated the effects of the experimental factors and the interactions between those factors. An optimization of the flocculation conditions was carried out. The results were analyzed using the software Minitab 15. It is used for calculating basic statistics and for simple estimation and hypothesis.

RSM regression

Response surface methods are used to examine the relationship between one or more response variables and a set of quantitative experimental variables or factors (Khuri & Cornell 1987). The factors considered in this study are the pH of the effluent (3, 6.5 and 10), the flocculant dose (1, 5 and 9 mg/L), the mixing flocculation speed (30, 75 and 120 rpm) and the mixing flocculation time (1, 5 and 9 min), whereas the experimental results to be evaluated are the decolorization, the COD removal, the turbidity abatement and the TSS of the treated effluent. The factors values are chosen from the effect study of each factors developed in the previous part.

The experimental surface plan to model is described in Table 3; its regression analysis by a quadratic model leads to the following equations:

\[
\text{COD removal} = 88.64 + 2.049\,\text{pH} + 0.601\,D + 0.345\,T + 0.0054 - 0.1301\,\text{pH} \times \text{pH} \\
- 0.0236\,D + D - 0.0314\,T \times T \\
- 0.000119\,S + S - 0.0182\,pH \times D \\
- 0.0289\,pH \times T - 0.00072\,pH \times S \\
- 0.0286\,D \times T + 0.00027\,D \\
+ S + 0.00363\,T \times S; (4)
\]

\[
\text{Decolorization} = -31.1 + 22.77\,\text{pH} + 11.15\,D \\
+ 6.40\,T + 0.042\,S - 1.315\,\text{pH} \times \text{pH} \\
- 0.591\,D + D - 0.334\,T \times T \\
- 0.00065\,S \times S - 0.442\,pH \times D \\
- 0.500\,pH \times T + 0.0056\,pH \times S \\
- 0.088\,D \times T - 0.0050\,D \times S \\
+ 0.0090\,T \times S; (5)
\]

\[
\text{Tur} = 35.5 + 14.76\,\text{pH} + 0.65\,D + 3.47\,T \\
- 0.804\,S - 1.045\,pH \times pH - 0.220\,D \\
+ D - 0.261\,T \times T + 0.00563\,S \\
+ S + 0.375\,pH \times D - 0.214\,pH \times T \\
+ 0.0159\,pH \times S - 0.047\,D \times T \\
+ 0.0100\,D \times S + 0.0014\,T \times S; (6)
\]

\[
\text{TSS (mg/L)} = 1.0 + 4.90\,\text{pH} + 0.90\,D + 1.09\,T \\
- 0.263\,S - 0.399\,pH \times pH - 0.158\,D \\
+ D - 0.202\,T \times T + 0.000895\,S \\
+ S + 0.107\,pH \times D + 0.039\,pH \times T \\
+ 0.0073\,pH \times S + 0.083\,D \times T \\
+ 0.0019\,D \times S + 0.0039\,T \times S; (7)
\]
where $\text{pH}$ is the pH of the effluent, $D$ (mg/L) is the flocculant dose, $S$ (rpm) is the mixing flocculation speed, ensure space between $T$ and (min) is the mixing flocculation time, and decolorization (%), COD removal (%) and Tur (%) were calculated, respectively, according to Equations (1)–(3), mentioned previously.

For the regression equation of the decolorization, COD removal, turbidity and TSS, it was found that the squared multiple correlation coefficients, $R^2$, were, respectively, 89.77%, 81.42%, 89.59% and 86.93%. So, we can conclude that the models obtained for the responses had a good predictability ($R^2 = 100\%$ represents a perfect predictability) (Ben Ticha et al. 2016).

### Variance analysis (ANOVA)

In order to determine the significant main and the interaction effects of the factors influencing the performance of the flocculation treatment using bio-product, an ANOVA was performed using Student’s $t$-test at $P < 0.05$ (Carmona et al. 2005). The $P$-value is the probability value that is used to determine the statistically significant effects in the mathematical model. The importance of the data can be judged by the $P$-values, with values closer to zero denoting greater significance. For 95% confidence level the $P$-value should be less than 0.05 for the effect to be considered statistically significant (Srinivasan & Viraraghavan 2010).

### Table 3 | Actual level of studied variables and results obtained for a surface design

<table>
<thead>
<tr>
<th>Run</th>
<th>pH</th>
<th>$D$ (mg/L)</th>
<th>$T$ (min)</th>
<th>$S$ (rpm)</th>
<th>COD removal (%)</th>
<th>Decolorization (%)</th>
<th>Tur (%)</th>
<th>TSS (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.5</td>
<td>5</td>
<td>5</td>
<td>75</td>
<td>98.404</td>
<td>97.744</td>
<td>70.0</td>
<td>14.8</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>30</td>
<td>96.146</td>
<td>88.404</td>
<td>52.0</td>
<td>8.4</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>5</td>
<td>9</td>
<td>75</td>
<td>96.541</td>
<td>76.808</td>
<td>52.0</td>
<td>9.2</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>1</td>
<td>5</td>
<td>75</td>
<td>96.069</td>
<td>69.446</td>
<td>56.0</td>
<td>9.6</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>120</td>
<td>96.191</td>
<td>75.532</td>
<td>42.0</td>
<td>4.4</td>
</tr>
<tr>
<td>6</td>
<td>6.5</td>
<td>9</td>
<td>9</td>
<td>75</td>
<td>97.197</td>
<td>89.360</td>
<td>61.0</td>
<td>11.6</td>
</tr>
<tr>
<td>7</td>
<td>6.5</td>
<td>5</td>
<td>5</td>
<td>75</td>
<td>98.385</td>
<td>95.936</td>
<td>70.0</td>
<td>14.8</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>5</td>
<td>9</td>
<td>75</td>
<td>96.226</td>
<td>76.383</td>
<td>43.0</td>
<td>3.2</td>
</tr>
<tr>
<td>9</td>
<td>6.5</td>
<td>9</td>
<td>5</td>
<td>30</td>
<td>98.315</td>
<td>96.383</td>
<td>84.0</td>
<td>18.8</td>
</tr>
<tr>
<td>10</td>
<td>6.5</td>
<td>1</td>
<td>9</td>
<td>75</td>
<td>97.226</td>
<td>86.383</td>
<td>58.0</td>
<td>7.2</td>
</tr>
<tr>
<td>11</td>
<td>6.5</td>
<td>5</td>
<td>9</td>
<td>30</td>
<td>96.584</td>
<td>77.574</td>
<td>82.0</td>
<td>19.4</td>
</tr>
<tr>
<td>12</td>
<td>6.5</td>
<td>1</td>
<td>1</td>
<td>75</td>
<td>97.010</td>
<td>75.890</td>
<td>58.0</td>
<td>7.4</td>
</tr>
<tr>
<td>13</td>
<td>6.5</td>
<td>5</td>
<td>1</td>
<td>120</td>
<td>97.226</td>
<td>86.383</td>
<td>68.0</td>
<td>8.2</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td>9</td>
<td>5</td>
<td>75</td>
<td>96.294</td>
<td>75.744</td>
<td>76.0</td>
<td>14.4</td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>75</td>
<td>95.860</td>
<td>41.276</td>
<td>46.0</td>
<td>5.6</td>
</tr>
<tr>
<td>16</td>
<td>6.5</td>
<td>1</td>
<td>5</td>
<td>120</td>
<td>97.331</td>
<td>86.510</td>
<td>60.0</td>
<td>8.2</td>
</tr>
<tr>
<td>17</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>30</td>
<td>97.247</td>
<td>85.532</td>
<td>78.0</td>
<td>15.0</td>
</tr>
<tr>
<td>18</td>
<td>6.5</td>
<td>9</td>
<td>5</td>
<td>120</td>
<td>98.781</td>
<td>97.404</td>
<td>83.2</td>
<td>19.2</td>
</tr>
<tr>
<td>19</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>75</td>
<td>95.434</td>
<td>68.936</td>
<td>48.0</td>
<td>4.8</td>
</tr>
<tr>
<td>20</td>
<td>6.5</td>
<td>5</td>
<td>5</td>
<td>75</td>
<td>98.444</td>
<td>96.744</td>
<td>70.0</td>
<td>14.8</td>
</tr>
<tr>
<td>21</td>
<td>6.5</td>
<td>9</td>
<td>1</td>
<td>75</td>
<td>98.809</td>
<td>84.510</td>
<td>64.0</td>
<td>6.5</td>
</tr>
<tr>
<td>22</td>
<td>10</td>
<td>5</td>
<td>1</td>
<td>75</td>
<td>97.369</td>
<td>97.383</td>
<td>69.0</td>
<td>8.6</td>
</tr>
<tr>
<td>23</td>
<td>3</td>
<td>9</td>
<td>5</td>
<td>75</td>
<td>97.104</td>
<td>72.340</td>
<td>45.0</td>
<td>4.4</td>
</tr>
<tr>
<td>24</td>
<td>6.5</td>
<td>5</td>
<td>9</td>
<td>120</td>
<td>98.594</td>
<td>89.361</td>
<td>65.0</td>
<td>8.6</td>
</tr>
<tr>
<td>25</td>
<td>6.5</td>
<td>5</td>
<td>1</td>
<td>30</td>
<td>97.832</td>
<td>81.065</td>
<td>86.0</td>
<td>21.8</td>
</tr>
<tr>
<td>26</td>
<td>6.5</td>
<td>1</td>
<td>5</td>
<td>30</td>
<td>97.054</td>
<td>81.914</td>
<td>68.0</td>
<td>9.2</td>
</tr>
<tr>
<td>27</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>120</td>
<td>96.569</td>
<td>76.170</td>
<td>78.0</td>
<td>15.6</td>
</tr>
</tbody>
</table>
The variance analysis of the decolorization, COD removal, the turbidity and TSS parameters are given, respectively, in Table 4.

According to Table 4, the ANOVA proves that, for the flocculation performance parameters, the regression models obtained (Equations (4)–(7)) were highly significant ($P$-value varied from 0.000 to 0.038). Moreover, there is a significant square effect and a significant linear effect ($P < 0.05$). So, it seems that the studied factors are statistically significant. However, the $P$-values of the interactions varied from $P = 0.072$ to $P = 0.923$. So, the factor interactions are statistically non-significant with 95% confidence level.

**Analysis of the main effects plot**

The main effects of each factor (pH, flocculant dose, mixing flocculation speed, and mixing flocculation time) on the flocculation treatment performance are shown in Figure 4(a)–4(d). A main effect occurs when the mean response changes across the levels of a factor. It is used to compare the relative strength of the effects across factors (Ben Ticha et al. 2013).

Analyzing the graphs of Figure 4, it seems that the behavior of these factors varies from one response to another. But, it is clear that the pH of the effluent was the most important variable for the flocculation treatment performance.

**Analysis of interactions plot**

The interaction effect plots were also studied and are presented in Figure 5(a)–5(d). The interaction between factors occurs when the change in response from the low level to the high level of one factor is different from the change in response at the same two levels of a second factor (Ben Ticha et al. 2013). From the graphs of Figure 5, Equations (4)–(7) and the ANOVA, it can be seen that there are significant interactions between all studied factors.

**Contour plot of response**

Contour plots, selected in such a way as to demonstrate the main effect of individual variables, together with those representing the most significant variable combinations,

<table>
<thead>
<tr>
<th>Responses</th>
<th>Source</th>
<th>DF</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decolorization (%)</td>
<td>Regression</td>
<td>14</td>
<td>2765.28</td>
<td>197.52</td>
<td>2.14</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Linear</td>
<td>4</td>
<td>742.96</td>
<td>185.74</td>
<td>2.01</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>Square</td>
<td>4</td>
<td>1647.98</td>
<td>411.99</td>
<td>4.47</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>6</td>
<td>374.34</td>
<td>62.39</td>
<td>0.68</td>
<td>0.072</td>
</tr>
<tr>
<td></td>
<td>Residual error</td>
<td>12</td>
<td>1106.83</td>
<td>92.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>26</td>
<td>3872.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COD removal (%)</td>
<td>Regression</td>
<td>14</td>
<td>21.54</td>
<td>1.53</td>
<td>7.52</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Linear</td>
<td>4</td>
<td>3.90</td>
<td>0.97</td>
<td>4.77</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>Square</td>
<td>4</td>
<td>14.11</td>
<td>3.52</td>
<td>17.26</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>6</td>
<td>3.52</td>
<td>0.58</td>
<td>2.87</td>
<td>0.057</td>
</tr>
<tr>
<td></td>
<td>Residual error</td>
<td>12</td>
<td>2.45</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>26</td>
<td>23.99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turbidity (%)</td>
<td>Regression</td>
<td>14</td>
<td>4114.84</td>
<td>293.92</td>
<td>7.38</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Linear</td>
<td>4</td>
<td>2176.4</td>
<td>544.24</td>
<td>13.66</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Square</td>
<td>4</td>
<td>1751.19</td>
<td>437.8</td>
<td>10.99</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>6</td>
<td>186.71</td>
<td>31.12</td>
<td>0.78</td>
<td>0.601</td>
</tr>
<tr>
<td></td>
<td>Residual error</td>
<td>12</td>
<td>478.15</td>
<td>39.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>26</td>
<td>4592.99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>Regression</td>
<td>14</td>
<td>548.39</td>
<td>39.17</td>
<td>2.86</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>Linear</td>
<td>4</td>
<td>275.66</td>
<td>68.91</td>
<td>5.03</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>Square</td>
<td>4</td>
<td>247.75</td>
<td>61.93</td>
<td>4.52</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>6</td>
<td>24.97</td>
<td>4.16</td>
<td>0.3</td>
<td>0.923</td>
</tr>
<tr>
<td></td>
<td>Residual error</td>
<td>12</td>
<td>164.49</td>
<td>13.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>26</td>
<td>712.88</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DF: degrees of freedom; Adj SS: adjusted sum of squares; Adj MS: adjusted mean square.
were obtained. These plots (Figure 6) show the variations of decolorization, COD removal, turbidity and TSS, as a result of selecting different values of two variables while the variables for the other variables are held constant. Analysis of the contour plots may be used to identify an optimized solution for the studied response.

From Figure 6, it can be seen that a pH of about 7.5, a flocculant dose between 6 and 9 mg/L, a mixing time between 4 and 6 min and a mixing speed of about 30 to 60 rpm are the optimum conditions for an efficient flocculation treatment.

Response optimization

The optimal conditions of the flocculation treatment with Vicia faba mucilage were predicted by the response optimizer tool of Minitab 15 software for maximized response. The results are given in Figure 7. The optimal level of the selected factors were as follows: pH of the effluent of about 7.08, flocculant dose of about 6.75 mg/L, flocculation mixing speed of about 30 rpm and flocculation mixing time of about 3 min, leading in theory to a decolorization equal to 96.84%, COD removal of about 98.55%, turbidity abatement of about 85.13% and TSS of about 18.9 mg/L with an overall desirability value equal to 93.2%.

Model validation

The validation experiment was performed to verify the accuracy of the model. Validation tests were carried out at the optimum conditions described in the previous section (pH of the effluent of about 7, flocculant dose of about 6.75 mg/L, flocculation mixing speed of about 30 rpm for 3 min).

The experiments were conducted in triplicate and the average value was calculated. In theory, the optimum values under these optimum conditions of the decolorization, COD removal, turbidity abatement and TSS were, respectively, 96.84%, 98.55%, 85.13% and 18.9 mg/L, whereas, the experimental values obtained were, respectively, 92.32%, 97.52%, 81.8% and 15.3 mg/L.
By the comparison of the mean values of the obtained and the predicted values, it is obvious that the model is validated.

Comparison of flocculation process performance

Natural flocculants, mainly polysaccharides, are considered environmentally friendly in comparison with inorganic and organic coagulants due to their biodegradability (Mishra & Bajpai 2006). However, a comparative study of two commercialized flocculants (EPENWATE EXP 31/1 and polyacrylamide A100PWG) and the Vicia faba mucilage was carried out and the results are given in Figure 8. As shown in Figure 8, the COD removal using the Vicia faba mucilage as a bio-flocculant was higher than that achieved by EPENWATE EXP 31/1 and polyacrylamide A100PWG. However, the decolorization and the turbidity of the effluent using the bio-agent were lower than those achieved by chemical products.

From the economic and ecological point of view, the flocculation process using the natural agent was better than treatment using commercial products.

In this regard, Table 5 compares the results obtained in this research to those obtained in other studies where natural flocculants were used.

The above results showed that the natural flocculants can be effectively employed in wastewater treatment. However, practically all reported studies took place under lab-scale conditions. The development of extraction processes, the characterization of the natural active ingredients, and application at pilot and full scale, as well as cost analysis, are necessary steps for the eventual application of natural flocculants.

CONCLUSION

From the present set of experiments, the flocculation process performance using a new bio-agent extract of Vicia faba
Figure 6 | Contour plots of the studied responses: (a) for decolorization; (b) for COD removal; (c) for turbidity; (d) for total suspended solids.

Figure 7 | Response optimization.

Figure 8 | Comparison of the flocculation treatment performance between natural and commercial flocculants.
faba was evaluated via the measurement of the decolorization, the COD removal, the turbidity abatement and the TSS after treatment. The modeling and optimization of flocculation conditions were carried out using ANOVA response. The optimal flocculation pH, dose of flocculant, flocculation mixing speed and flocculation mixing time were, respectively, about 7, 6.75 mg/L, 30 rpm and 3 min. At the optimal conditions, the decolorization, the COD removal, the turbidity abatement and the TSS were, respectively, 92.32%, 97.52%, 81.8% and 15.3 mg/L. The comparative study with commercial flocculants (EPENWATE EXP 31/1, polyacrylamide A100PWG) showed that the Vicia faba mucilage had a good flocculation performance. Thus, flocculation using Vicia faba mucilage as bio-flocculant for pollution removal was proved to be a simple and efficient treatment from an economic and ecological point of view. In this paper, a new bio-agent and abundant product was found for textile wastewater treatment with a low cost.

ACKNOWLEDGEMENTS

The authors are particularly grateful to Mr Mohamed Touzi the General Director of SITEX (Société Industrielle des Textiles); Mr Moez Kechida, the development engineer in SITEX; and all the technical and administrative staff of this company for their assistance and valuable contribution.

REFERENCES


Bouatay, F. & Mhenni, F. 2014 Use of the cactus cladodes mucilage (Opuntia ficus indica) as an eco-friendly flocculants: process


Fan, L., Zhou, Y., Yang, W., Chen, G. & Yang, F. 2008 Electrochemical degradation of aqueous solution of Amaranth azo dye on ACF under potentiostatic model. Dyes Pig. 76, 440–446.


