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Evaluation of the potentiality of *Vicia faba* and *Opuntia ficus indica* as eco-friendly coagulants to mitigate *Microcystis aeruginosa* blooms

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ABSTRACT

This study aims to explore the potentiality of *Vicia faba* seeds and *Opuntia ficus indica* cladodes, as eco-friendly coagulants, to sanitize water from *Microcystis aeruginosa* blooms. The effect of factors influencing coagulation–flocculation activity were studied, namely, coagulant dose (5–20 mg/L), pH (5–8), rapid-mixing speed (100–250 rpm) and slow-mixing (presence or absence). Levels used for each factor, were studied by using the experimental research methodology. The efficiency of the studied natural coagulants was compared to that of chemical coagulants (aluminum sulfate and ferric chloride). The assays were conducted using the standard jar test method. Indeed, turbidity, optical density, chlorophyll a and carotenoids reductions were maximal (more than 85%) with 5 and 10 mg/L doses, under the presence of slow-mixing speed of 40 rpm and rapid-mixing speed of 200 rpm. The favorable pH to perform the treatment was confirmed at pH 5. The analysis of extracts by chemical assays and Fourier-transform infrared spectral analysis, confirmed the presence of total sugar, phenols, proteins, and flavonoids groups, which are known by their coagulating potential. Results demonstrated that these two natural coagulants could be used as an eco-friendly alternative to chemical coagulants to decontaminate water polluted with cyanobacteria blooms.

Keywords: *Microcystis aeruginosa*; *Vicia faba*; *Opuntia ficus indica*; Coagulation–Flocculation; Experimental design

1. Introduction

The proliferation of harmful algal blooms (HABs) has become a major problem, especially at water treatment plants [1]. Algal proliferation causes a real change in turbidity, pH, taste, odor, and amount of organic matter in treated water [2] and can easily block the deep-bed filter, which makes it necessary to increase the frequency of backwashing [3]. The presence of microalgae in high concentrations is also related to the problems of cyanotoxins (hepatotoxins, neurotoxins, cytotoxins, and dermatoxins) release due to the cell decay and breakdown [4].

Cyanobacteria are among microalgae that pose those problems for water utilities worldwide [5]. They are the first

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oxygen-evolving group of photosynthetic prokaryotes and commonly referred to as blue-green algae. Excessive growth of cyanobacteria such as *Microcystis* sp. produces water-blooms [6], which is a serious constraint for sustainable and multiple use of water resources [7].

A variety of processes such as filtration, coagulation–flocculation, sedimentation [8], prechlorination [9,10] and ozonation [9] have been developed to reduce noxious effects of cyanobacteria [11]. In water and wastewater treatment, coagulation technique is widely known and used due to its several advantages, including cost reduction, easy operation, and good performance [12]. Therefore, chemical coagulants and flocculants are commonly used to remove water color and turbidity, in the form of suspended and colloidal material [13]. Also, they are used to destabilize and remove algal blooms [10,14]. In addition, they have been demonstrated that coagulation–flocculation process is most effective to remove trace metal from humus effluent [15] and it is among the techniques considered to be effective and low cost to remove arsenic from groundwater [16]. Some of the most commonly used coagulants are aluminum salts, and ferric chloride due to their high removal efficiency [17,18].

Adding a coagulant that generally has a positive charge causes neutralization of the electrostatic face potential of the particles, which destabilize particles and they stick to each other on contact forming solids called "flocs" [19]. Rapid-mixing for a few seconds is necessary to guarantee a homogeneous dispersion of the coagulant and to increase the probability of contact of all the particles, followed by adding a flocculant in the slow-mixing for at least 15 min, which promotes the formation of flocs [20]. After coagulation–flocculation process, cyanobacterial cells are transferred to the accumulated flocs, in which cyanobacteria are prone to lose their viability due to cell damage, and finally their elimination [21].

Despite their performance in term of pollution depletion, these coagulants present several disadvantages; such as, the production of harmful high quantities of sludge, the requirement of pH, and alkalinity adjustment, the high operation costs, and the ineffectiveness at low water temperatures [17,22]. In addition, some of those inorganic coagulants are not biodegradable [23] and are usually found in the wastewater after treatment, which may cause serious environmental problems [24]. Moreover, the chemical stress induced during the coagulation process destined to remove cyanobacteria may lead to cell lysis, which results in the release of intracellular organic matter, including cyanotoxins and consecutively the deterioration of water quality [25]. Consequently, further treatments are required after the coagulation–flocculation process to have clear water and less of extracellular toxins [26,27].

To overpass those problems, an alternative and eco-friendly method based on the use of natural coagulants has become widely explored [17,28]. Natural coagulants have the advantages of being biodegradable, cost effective, and originated from natural an available materials and harmless to public health and environment [29,30]. Furthermore, plant-based natural coagulants are non-corrosive and can produce an amount of sludge five times lower than that produced by chemical coagulants, their application decreases the operation and handling costs of the potable water production and therefore maintain sustainable development of water purification process [31,32].

Many plant materials were identified as a source of natural coagulants to remove turbidity of water [13,33–35]. Among others, seeds of *Moringa oleifera* have been reported as one of the most effective primary coagulants for water treatment [17,36]. They have been applied in water clarification, especially for high turbid waters (>100 NTU) [37] and as a cyanobacterial removal agent [38]. Faba bean seeds (*Vicia faba*) and cactus cladodes (*Opuntia ficus indica*) have been also used for water clarification as natural coagulants to remove suspended solids [39,40]. Studies have shown that cactus has similar coagulating potential as seeds of *M. oleifera*. To our knowledge, these two plants have not been studied for the removal of cyanobacterial blooms.

The aim of this study is to investigate the effects of faba bean seeds (*V. faba*) and cactus cladodes (*O. ficus indica*), as natural coagulants to remove cyanobacterial blooms caused by *Microcystis aeruginosa*. These two types of plants have been chosen for their abundance in Morocco. Faba bean is one of the most cultivated annual plants; its raw material is very abundant and has the most important legume crop; whereas, cactus is considered as a wild plant and characterized by its succulent property, which is rich in polysaccharides. The coagulating potential of these two plants and the effects of different parameters involved in the coagulation–flocculation process were assessed in comparison with chemical coagulants ($Al_2(SO_4)$ and $FeCl_3$) to remove cyanobacteria.

2. Material and methods

2.1. Cyanobacterial culture

The cyanobacterial species used in this study is *M. aeruginosa*, selected because of its prevalence in algal blooms and its negative impact on water quality and treatment, which releases toxins and odor substances into water [41].

The *M. aeruginosa* was sampled during bloom occurrence at Lalla Takerkoust reservoir (LTR) (area of Marrakech), isolated and maintained since October 2015 at a room culture in batch system on Z8 medium, under optimal growth conditions; 63 μmol photons/m^2/s with a light dark cycle of 15/9 h at 26°C ± 2°C. *M. aeruginosa* complex from (LTR) was known by its toxic potential [42,43].

2.2. Preparation of algal suspension

In order to assess the coagulation–flocculation activity of the studied extracts, an algal suspension was artificially prepared by inoculating distilled water with an exponentially growing *M. aeruginosa* culture. The strain of *M. aeruginosa* was cultivated in autoclave-sterilized glass flasks (5 L) to obtain high biomass on liquid Z8 medium for 10 d under the optimal growth conditions mentioned above. After inoculation with *M. aeruginosa*, the cell density of the test mixture was measured to about 10^6 cells/mL. The mixture was prepared to stimulate high turbidity water following a proliferation of cyanobacteria. After being prepared, the samples were subjected to coagulation–flocculation process.

2.3. Preparation of natural coagulants

Natural coagulants were extracted from seeds of faba bean (*V. faba*), according to the method described by Kukić et al. [39] with a slight modification. Dry bean seeds were crushed into a fine powder and sieved through 0.5 mm sieve. One gram of sieved powder was suspended in 100 mL of NaCl solution (1 M) for extraction, this concentration was chosen based on preliminary tests. Subsequently, the suspension was stirred for 10 min, in order to extract active coagulants, and then filtered through 0.45 μm cellulose nitrate membrane filter. The filtrates obtained were stored at 5°C until further use.

Concerning cactus cladodes (*O. ficus indica*), the extraction of active components was performed by using the following procedure: cactus cladodes were sliced, washed, and dried at 100°C for 2 h and finally the dried cladodes were powdered and sieved through 0.2 mm sieve. One gram of this powder was suspended in 100 mL of NaCl (1 M) solution for extraction, this concentration was chosen based on preliminary tests. Then, the suspension was stirred for 15 min and left to rest for 15 min and therefore the supernatant liquid was used as coagulant [44].

2.4. Experiments design of screening study

Several parameters influence the coagulation–flocculation activity, such as pH, electrolyte dose, coagulant dose, agitation, initial water turbidity, temperature, composition of water, mixing speed, and settling time [39,45,46]. According to several researches, pH, and coagulant dose have the most important effect [45]. For this purpose, preliminary tests were carried out in order to determine the optimal range of pH and coagulant dose that will allow a maximum removal of *M. aeruginosa* by coagulation–flocculation.

Then, the treatment efficacy of water contaminated with *M. aeruginosa* using natural coagulants (faba bean and cactus cladodes extracts) and chemical coagulants (Al_2SO_4, $9H_2O$, and $FeCl_3$) were evaluated by application of the experimental research methodology. This method allows a complete study of the effects of all parameters involved in a given process, while providing maximum information with a minimum of experiments [47,48]. The results were subjected to statistical analysis to assess the relative significance of the main factors affecting the coagulation–flocculation as evaluated from the experimental results.

In the present study, the effect of agitation speed, type and coagulant dose, presence and absence of flocculant (polyelectrolyte), and pH were studied using an asymmetric factorial matrix of six factors including four factors at four levels and two factors at two levels (Table 1). A total of 16 experiments was performed with two repetitions (Table 2). The screening experience for several factors allows the estimation of k factors in N experiments where $k = 6$ and $N = 16$ according to a polynomial empirical model of first degree. This model will reflect the effect of each of the six factors on the four responses: turbidity, chlorophyll a, carotenoids, and optical density and it can be written as described by Eq. (1):

$$Y = b_0 + b_1A \times (X_1A) + b_2A \times (X_2A) + b_3A \times (X_3A) + b_3B \times (X_3B) + b_3C \times (X_3C) + b_4A \times (X_4A) + b_4B \times (X_4B) + b_4C \times (X_4C) + b_5A \times (X_5A) + b_5B \times (X_5B) + b_5C \times (X_5C) + b_6A \times (X_6A) + b_6B \times (X_6B) + b_6C \times (X_6C) \tag{1}$$

Table 1
Factors studied and experimental field

	Factors	Number of levels	Levels
U_1	Slow-mixing speed	2	Absence (Abs) Presence (Pres)
U_2	Flocculant	2	Absence (Abs) Presence (Pres)
U_3	Coagulant	4	Bean extract Cactus extract $Al_2(SO_4)$ $FeCl_3$
U_4	Dose (mg/L)	4	5 10 15 20
U_5	pH	4	5 6 7 8
U_6	Rapid-mixing speed (rpm)	4	100 150 200 250

Table 2
Experimentation plan

Number of experiments	Slow-mixing speed	Flocculant	Coagulant	Dose (mg/L)	pH	Rapid-mixing speed
1	Abs	Abs	Bean extract	5	5	100
2	Abs	Abs	Cactus extract	10	6	150
3	Abs	Abs	$Al_2(SO_4)$	15	7	200
4	Abs	Abs	$FeCl_3$	20	8	250
5	Pres	Abs	Bean extract	10	7	250
6	Pres	Abs	Cactus extract	5	8	200
7	Pres	Abs	$Al_2(SO_4)$	20	5	150
8	Pres	Abs	$FeCl_3$	15	6	100
9	Abs	Pres	Bean extract	15	8	150
10	Abs	Pres	Cactus extract	20	7	100
11	Abs	Pres	$Al_2(SO_4)$	5	6	250
12	Abs	Pres	$FeCl_3$	10	5	200
13	Pres	Pres	Bean extract	20	6	200
14	Pres	Pres	Bean extract	15	5	250
15	Pres	Pres	$Al_2(SO_4)$	10	8	100
16	Pres	Pres	$FeCl_3$	5	7	150

where Y is the studied response, X_i is the investigated factor (i varies from 1 to 6), A is the domain delimited by levels 1 and 2 of the factor X_i, B is the domain delimited by levels 2 and 3 of the factor X_i, b_iA is the X_i effect in domain A and b_iB is the X_i effect in the domain B.

The studied variables, with their respective ranges of values, were chosen on the basis of data from the literature and preliminary experiments. Matrix generation and data processing were performed using the NEMRODW software (new efficient methodology for research using optimal design from LPRAI, Marseille, France) [49].

2.5. Coagulation–flocculation experiments

Coagulation–flocculation experiments were performed in jar-test apparatus with a series of six 500 mL beakers containing the algal suspension and coagulants (natural extracts and chemical coagulants). For each experiment, an appropriate amount of coagulant was added to the algal suspension under rapid-maxing (during 2 min) to ensure the good dispersion of reagents and the destabilization of particles, followed by the addition of flocculant (polyelectrolyte) under slow-mixing (during 40 min) to promote the contact of the contiguous particles and avoid the breaking of formed flocs. The coagulation activity was calculated using Eq. (2) according to Kukić et al. [39].

$$\text{Cocagulation activity }(\%) = \frac{(T_i - T_f)}{T_i} \times 100 \tag{2}$$

where T_i and T_f (NTU) are the turbidity of the water sample before and after the test, respectively.

2.6. Analytical methods and parameters evaluation

The effectiveness of the coagulation–flocculation by natural coagulants (faba bean and cactus cladode extracts) and chemical coagulants (Al_2SO_4 and $FeCl_3$) was evaluated by measuring the optical density at 750 nm, the turbidity using a TN-100/T-100 USA turbidimeter, and the concentration of chlorophyll a and carotenoids before and after each experiment.

Chlorophyll a and carotenoids were measured according to the protocol developed by Wang et al. [4], their concentrations were calculated based on the method described by Lichtenthaler and Wellburn [50]. A volume of 10 mL of each culture was centrifuged for 15 min at 6,000 rpm. The pellet was recovered in 10 mL of boiling ethanol (95%). The three replicas were incubated at 4°C for 45 h. Afterwards, another centrifugation for 5 min at 5,000 rpm was carried out to remove the pellet formed. Then, supernatants were spectrophotometrically read at different wavelengths: 470, 649, 663, and 665 nm using Eq. (3). The pigment concentration was expressed in µg/mL, according to Eq. (4).

$$(\text{Chlorophylla}) = 13.95 \times \text{DO}_{665} - 6.88 \times \text{DO}_{649} \tag{3}$$

$$(\text{Carotenoids}) = \left[\frac{(1000 \times \text{DO}_{470}) - (2.05\,Ch - a)}{229}\right] \tag{4}$$

The general composition of the natural extract was identified to evaluate their potential use as natural coagulants. The following parameters: total sugar [51], proteins [52], total phenols using the Folin–Ciocalteu method [53], total tannins using the Folin–Denis [54], total flavonoids [55], and content of phytic acid [56].

Fourier transform infrared spectrophotometer (FTIR) spectral analysis was also used for the analysis of extracts. The spectral analysis was recorded in IRAffinity-1 spectrometer in the region of 400–4,000 cm^{-1}. The samples were prepared with lyophilized extracts of faba bean and cactus cladodes added by KBr under high pressure.

2.7. Statistical analysis

Statistical analysis was performed by SigmaPlot software and Microsoft Excel 2013. All experiments were done in three replicates. The results are expressed as mean ± standard deviation (SD). One-way ANOVA with the Tukey test was used to analyze the growth and physiological parameters. The significance of the results is compared at $\alpha < 0.05$.

3. Results

3.1. Tests of screening study: efficiency removal

Experimental design was used to study the effect of mixing speed, type and dose of coagulant, flocculant addition, and pH on the reduction of four parameters (turbidity, optical density, chlorophyll a, and carotenoids concentrations) of water contaminated with *M. aeruginosa*. The coagulation–flocculation was performed using natural coagulants (faba bean and cactus cladodes extracts) and chemical coagulants (Al_2SO_4, $FeCl_3$). The experimental responses (expressed as % reduction of each measured parameter) corresponding to the 16 performed experiments are shown in Table 3, while the graphical representations of the effect of factors corresponding to each response are illustrated in Figs. 1a–4a in which the size of the histogram is proportional to the intensity of each effect. The limits of significance of factors, at 99% ($p < 0.01$) and 95% ($p < 0.05$), are represented by dashed lines in Figs. 1b–4b, which depicts the differences in the weight of the different levels for each parameter response. A positive value is related to a synergetic and favored effect of factors, while a negative value indicates an antagonistic or a negative effect [57].

The theoretical results showed a remarkable effect for reducing turbidity, optical density, and chlorophyll a and carotenoids contents. The effect of slow-mixing speed (*A*1–2) on all studied responses was statistically significant ($p < 0.01$). The absence of slow-mixing influences unfavorably the studied responses since it had a negative coefficient value, which means the use of the slow-mixing improves the efficient removal of each parameter response.

Rapid-mixing speed was also statistically significant and showed a negative effect on the reduction of turbidity, optical density, chlorophyll a, and carotenoids when decreasing its values from 150 to 100 rpm (*F*1–2) (p < 0.05), from 200 to 100 rpm (*F*1–3) ($p < 0.01$), and from 200 to 150 rpm (*F*2–3) ($p < 0.01$). The effect of this parameter became positive from 250 to 100 rpm (*F*1–4), from 250 to 150 rpm (*F*2–4) ($p < 0.01$) and from 250 to 200 rpm (*F*3–4) ($p < 0.01$). From these results, it can be concluded that, comparatively to other tested speeds, the application of a rapid-mixing speed of 200 rpm can lead to the higher removal efficiency of all parameters response.

The coagulant type was statistically significant and showed also a double effect on the recoveries of all experimental responses. The results showed that the use of $Al_2(SO_4)$ instead of $FeCl_3$ allowed for an enhanced removal efficiency of all the measured responses; as evidenced by the positive value of *C*3–4 coefficient. $Al_2(SO_4)$ was also found as more efficient than faba beans (*C*1–3) and cactus cladodes (*C*2–3), while the comparison between these two natural extracts showed that the higher coagulation activity was observed for the faba beans extract (*C*1–2). According to these results, $Al_2(SO_4)$ as a chemical coagulant and faba beans extract as a natural coagulant could be effectively used to remove the chlorophyll a and carotenoids and reduce the turbidity of the contaminated water by coagulation–flocculation process.

The pH effect on the coagulation–flocculation activity was evaluated at values varying from 5 to 8. The results showed that at all conditions, the higher efficiency of the coagulation–flocculation process was obtained at lower pH values; the decrease of pH from 8 to 5 (*E*1–4), from 8 to 7 (*E*3–4), from 8 to 6 (*E*2–4), from 6 to 5 (*E*1–2) and from 7 to 5 (*E*1–3) affect positively the removal efficiency of all the measured responses. The negative effect was observed only when the pH decreased from 7 to 6 (*E*2–3). For all responses, the results showed that pH 5 was significantly the optimal pH for an efficient coagulation–flocculation.

Different concentrations (5, 10, 15, and 20 mg/L) were used to evaluate the effect of the coagulant dose on the coagulation–flocculation activity. The results revealed the existence of an optimal coagulant concentration at which the removal efficiency of all responses is maximized. As shown in Figs. 1–4, the higher removal efficiencies for all responses are observed at the coagulant dose of 10 mg/L. Increasing or decreasing the coagulant concentration above or less than this optimal limit may lead to the destabilization of the formed flocs and then to the decrease of the coagulation–flocculation activity [58]. In terms of the presence or absence of flocculant, the results showed no significant difference between these two levels (*B*1–2) for all studied responses.

In the conclusion of this screening study performed by the application of the experimental research methodology, the most significant reduction (more than 85%) of turbidity, optical density, chlorophyll a, and carotenoids concentrations, was recorded at pH 5 under a rapid-mixing speed of 200 rpm, in the presence of slow-mixing speed and by using faba bean extract or Al_2SO_4 as coagulant at 10 mg/L and without the use of flocculant. The experimental validation of the above results was carried out by three additional coagulation–flocculation experiments under the optimal conditions.

3.2. Experimental validation and comparison with chemical coagulants

From the results discussed above, it was shown that coagulation–flocculation using both natural coagulant and chemical coagulants was more efficient at a coagulant dose of 10 mg/L, rapid-mixing speed of 200 rpm, a pH of 5. The slow-mixing step was found improving the removal efficiency, while no flocculant addition was needed. To validate these results, a supplement experiment was conducted

Table 3
Designed experiments and results obtained from each response of experimental design

Number of experiments	Slow-mixing speed	Flocculant	Coagulant	Dose (mg/L)	pH	Rapid-mixing speed	Turbidity reduction (%)	Optical density reduction (%)	Chlorophyll a reduction (%)	Carotenoids reduction (%)
1	Abs	Abs	BE	5	5	100	83.85	80.26	78.49	62.15
2	Abs	Abs	CE	10	6	150	78.32	77.16	63.1	51
3	Abs	Abs	$Al_2(SO_4)$	15	7	200	87.5	84.25	86.76	73
4	Abs	Abs	$FeCl_3$	20	8	250	38.84	41.54	38.88	31.23
5	Pres	Abs	BE	10	7	250	96.79	96.73	91.21	80.5
6	Pres	Abs	CE	5	8	200	95.98	98.59	92.46	82.6
7	Pres	Abs	$Al_2(SO_4)$	20	5	150	98.65	100	94	84
8	Pres	Abs	$FeCl_3$	15	6	100	86.34	84.71	80.50	68
9	Abs	Pres	BE	15	8	150	59.2	60.8	50.58	37.85
10	Abs	Pres	CE	20	7	100	51.98	52.76	43.7	30.67
11	Abs	Pres	$Al_2(SO_4)$	5	6	250	69.47	70.99	68.14	53.2
12	Abs	Pres	$FeCl_3$	10	5	200	89.69	89.44	91.36	82
13	Pres	Pres	BE	20	6	200	96.81	100	93	83
14	Pres	Pres	BE	15	5	250	95.34	100	92.3	82.2
15	Pres	Pres	$Al_2(SO_4)$	10	8	100	98.1	100	94.3	87
16	Pres	Pres	$FeCl_3$	5	7	150	98.26	98.27	93.68	84.4

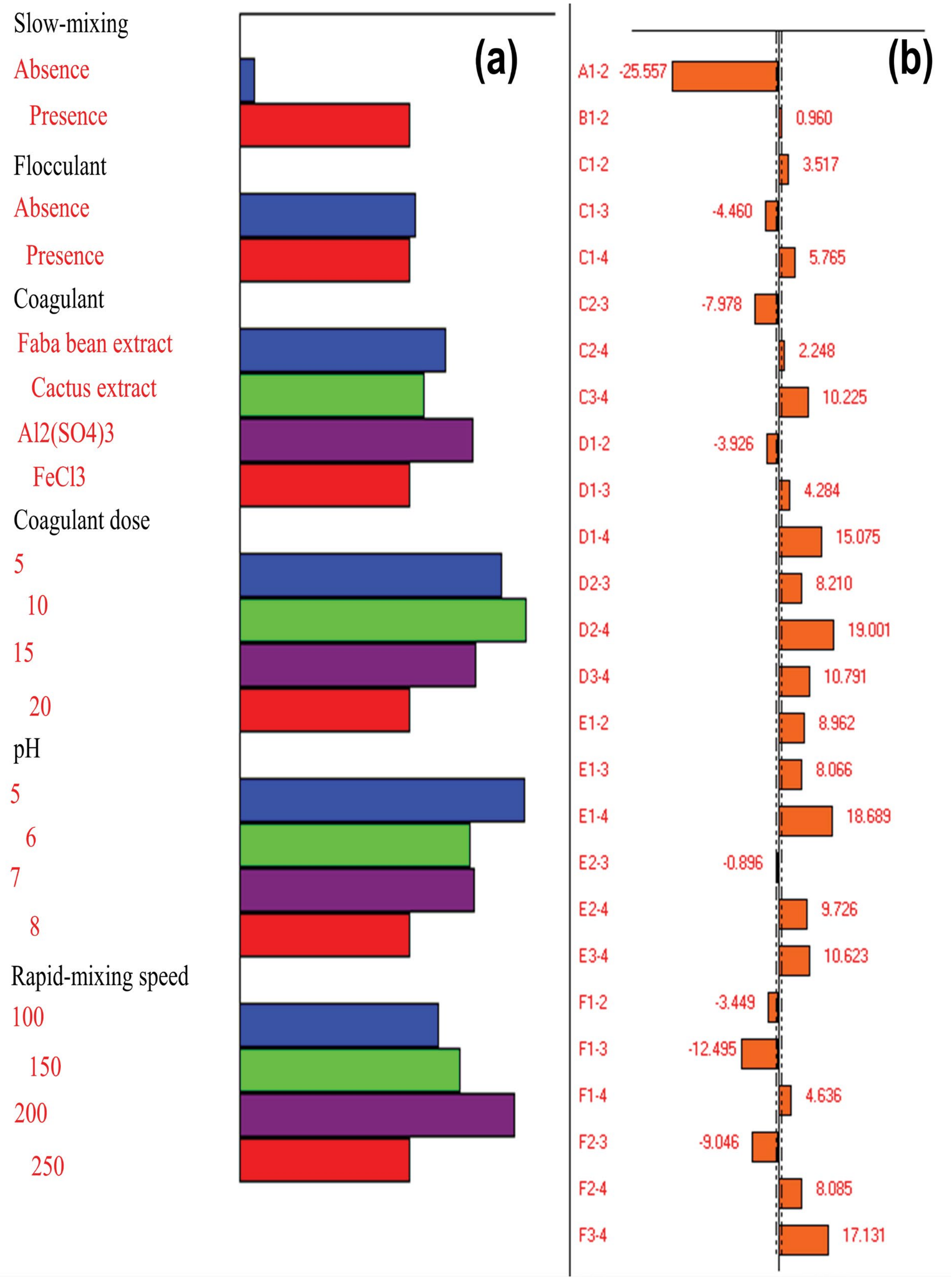

Fig. 1. Efficiency removal of turbidity response. (a) Factor effects on coagulation–flocculation activity and (b) differences in the weights of the different levels.

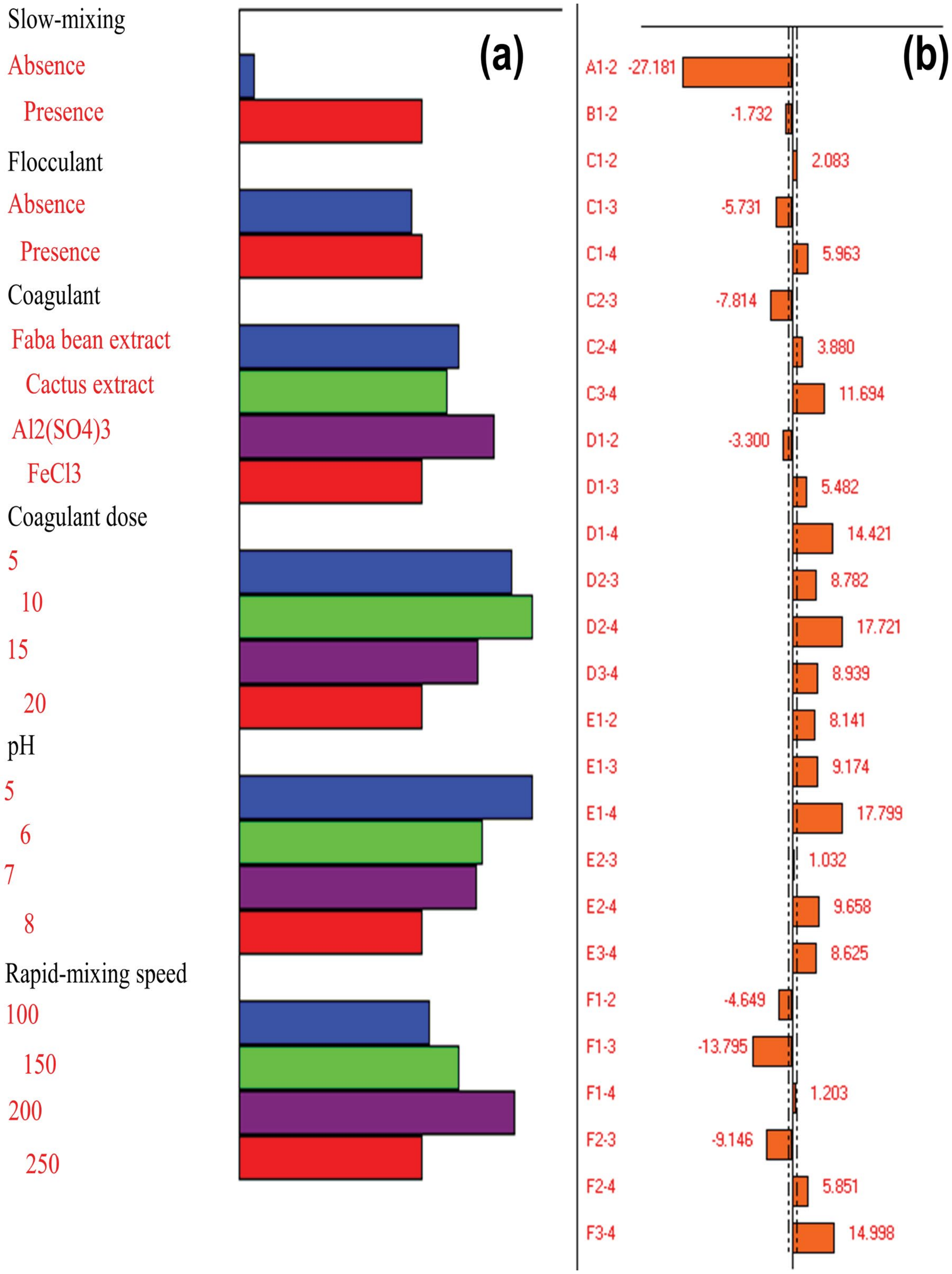

Fig. 2. Efficiency removal of optical density response. (a) Factor effects on coagulation–flocculation activity and (b) differences in the weights of the different levels.

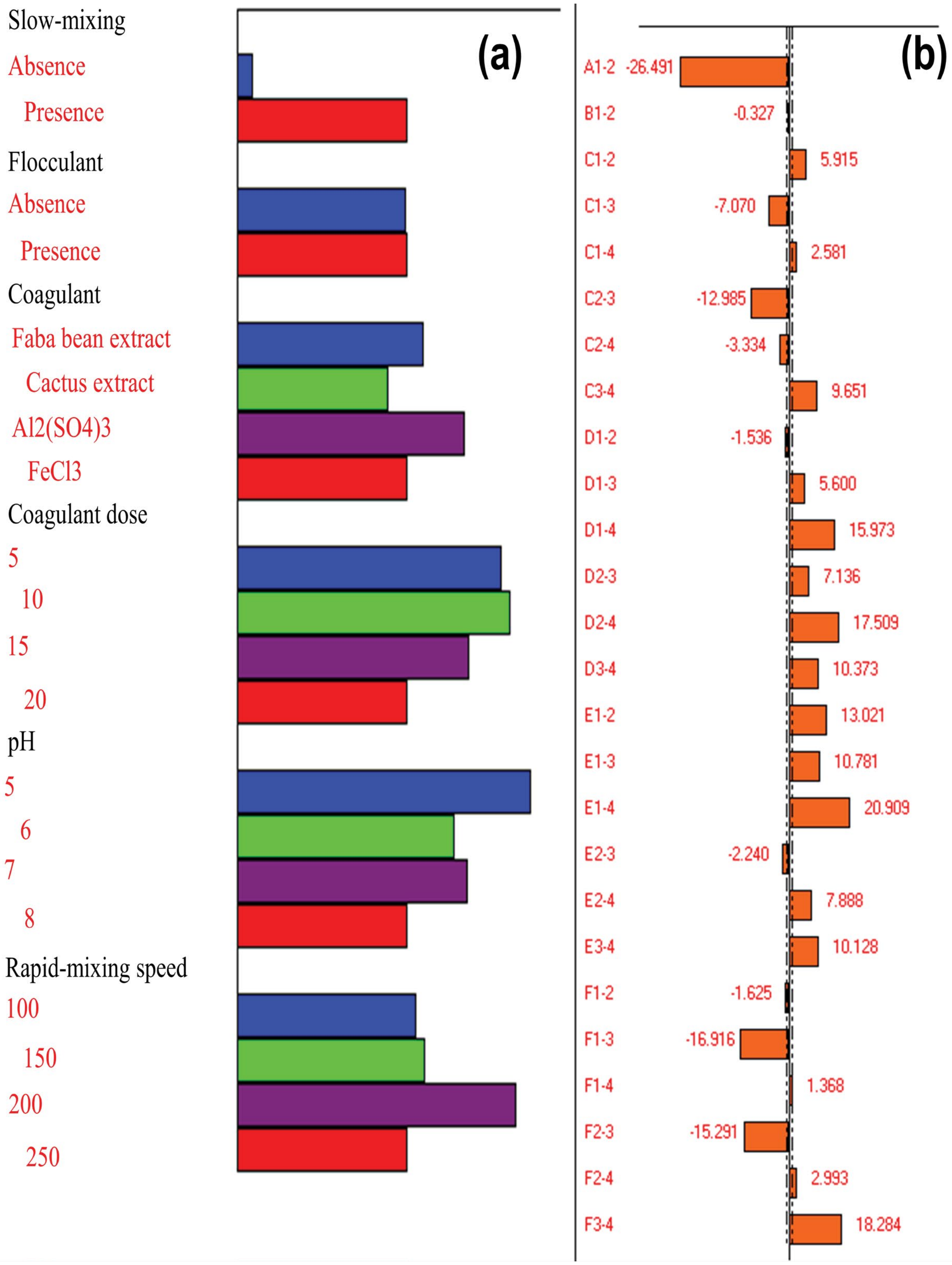

Fig. 3. Efficiency removal of chlorophyll a response. (a) Factor effects on coagulation–flocculation activity and (b) differences in the weights of the different levels.

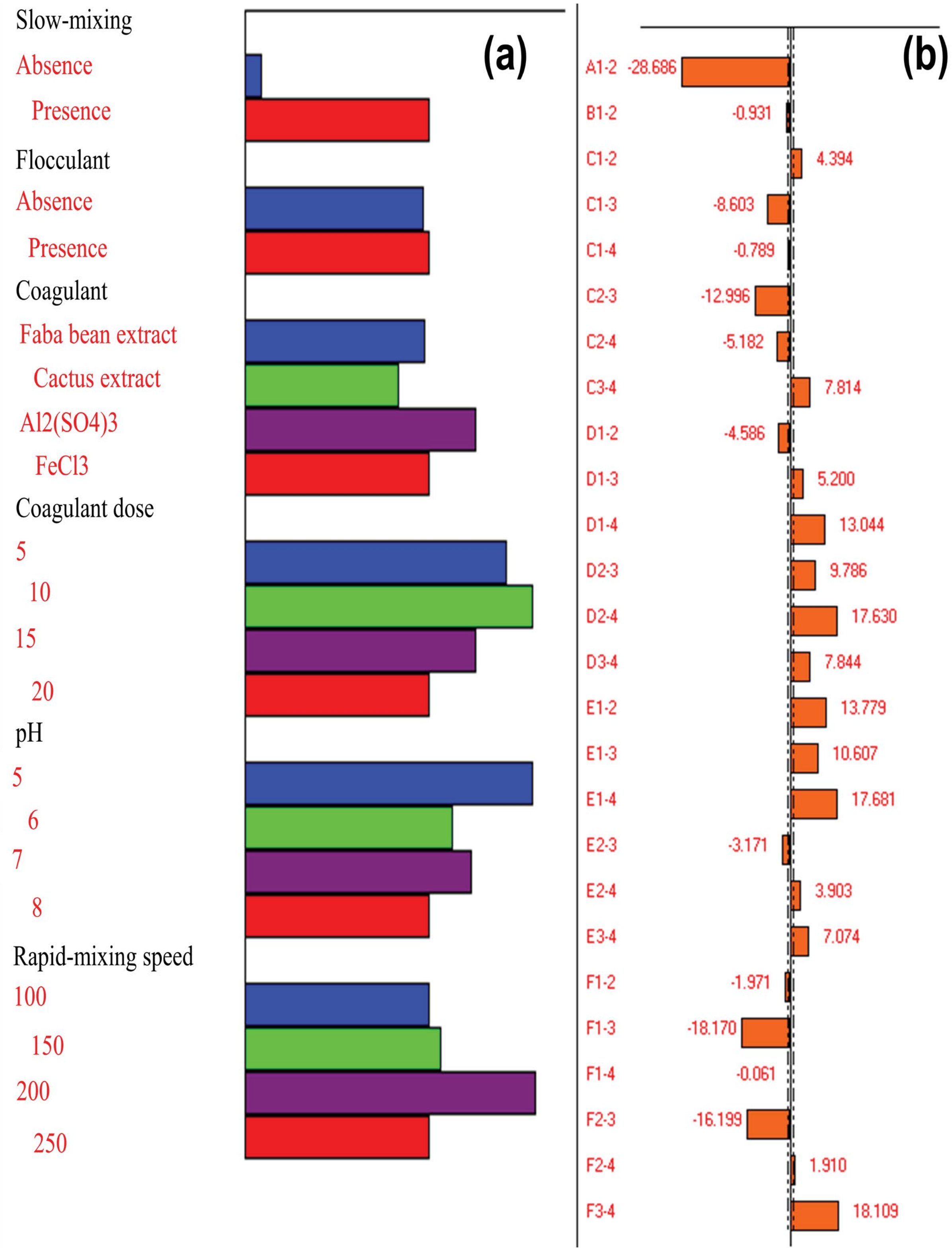

Fig. 4. Efficiency removal of carotenoids response. (a) Factor effects on coagulation–flocculation activity and (b) differences in the weights of the different levels.

under the conditions described above (all of coagulants have been tested). The results of the experimental validation test are presented in Fig. 5. The validation test was carried out in triplicate to verify the optimization levels of coagulation–flocculation activity.

From the obtained results, natural coagulants (*V. faba* and *O. ficus indica*) and chemical coagulants (Al_2SO_4 and $FeCl_3$) allowed for a percentage removal more than 85% for all the studied responses. Better coagulation was achieved with Al_2SO_4, followed by faba bean, cactus cladodes, and finally by $FeCl_3$. Statistical analysis showed a significant difference ($p < 0.001$ and 0.05) between the different types of coagulants, except between *V. faba* and *O. ficus indica*, for which no significant difference was observed. These results confirm the potential of *V. faba* and *O. ficus indica* as natural coagulants to remove the cyanobacterium *M. aeruginosa* by coagulation–flocculation under the experimental conditions predicted by the experimental research methodology performed in the present study.

3.3. FTIR characterization of V. faba and O. ficus indica extracts

The characterization of cactus cladodes and faba bean extracts by FTIR was performed to differentiate the material and provide information on the nature of functional groups on the surface of the natural coagulants tested. The FTIR spectrum in the range of 400–4,000 cm^{-1} of *V. faba* and *O. ficus indica* extracts are shown in Fig. 6a and b, respectively. The strong stretches observed around 3,300–3,500 cm^{-1} may be due to the OH polymeric stretching vibration of water and the amine stretching vibration, which indicates alcohols and phenols groups. The peaks at 1,084 and 1,082 cm^{-1} for *V. faba* and *O. ficus indica*, respectively, were due to C–O which confirmed the presence of alcohols and phenols groups. The peaks around 1,450–1,650 cm^{-1} were due to the presence of C=C, indicating the presence of an aromatic group. Certainly, acute peaks above 3,000 cm^{-1} confirmed the presence of the aromatic group. Hence, the FTIR spectra reveal that *V. faba* and *O. ficus indica* extracts mainly carries aromatic, alcohols, and phenols groups.

3.4. Analyses of the extracts of natural coagulants

The extracts obtained from *V. faba* and *O. ficus indica* were analyzed in order to determine in general the active compounds responsible for the coagulation–flocculation activity. Results of analyses are presented in Table 4, and data presented are expressed as mean ± standard deviation (SD) of three analyses.

The results showed that sugar and phenols in investigated extracts of faba bean seeds and cactus cladodes were present in higher concentrations than all other constituents. However, extract of faba bean seeds contained higher concentration of proteins and phytic acid than extract of cactus cladodes. On the contrary, amounts of extracted phenolics, flavonoids, total sugar, and tannins from cactus cladodes were present in greater amount as compared to faba bean seeds.

4. Discussion

We initially used a screening study to evaluate the influence of different parameters (mixing-speed, type and coagulant dose, flocculant (polyelectrolyte) addition, and pH), for coagulation–flocculation activity on water contaminated by *M. aeruginosa*, using natural coagulants (faba bean and cactus cladodes extracts) in comparison with chemical coagulants (Al_2SO_4, $FeCl_3$). The results of

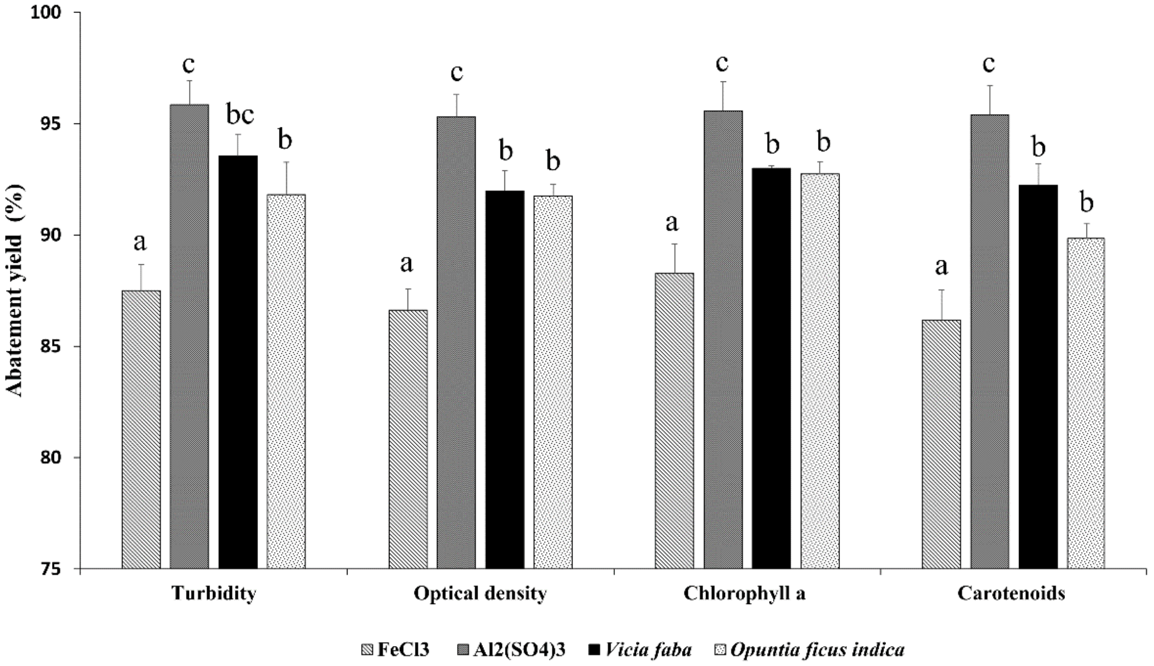

Fig. 5. Abatement rate in validation test using four parameters responses with comparison between chemical and natural coagulants.

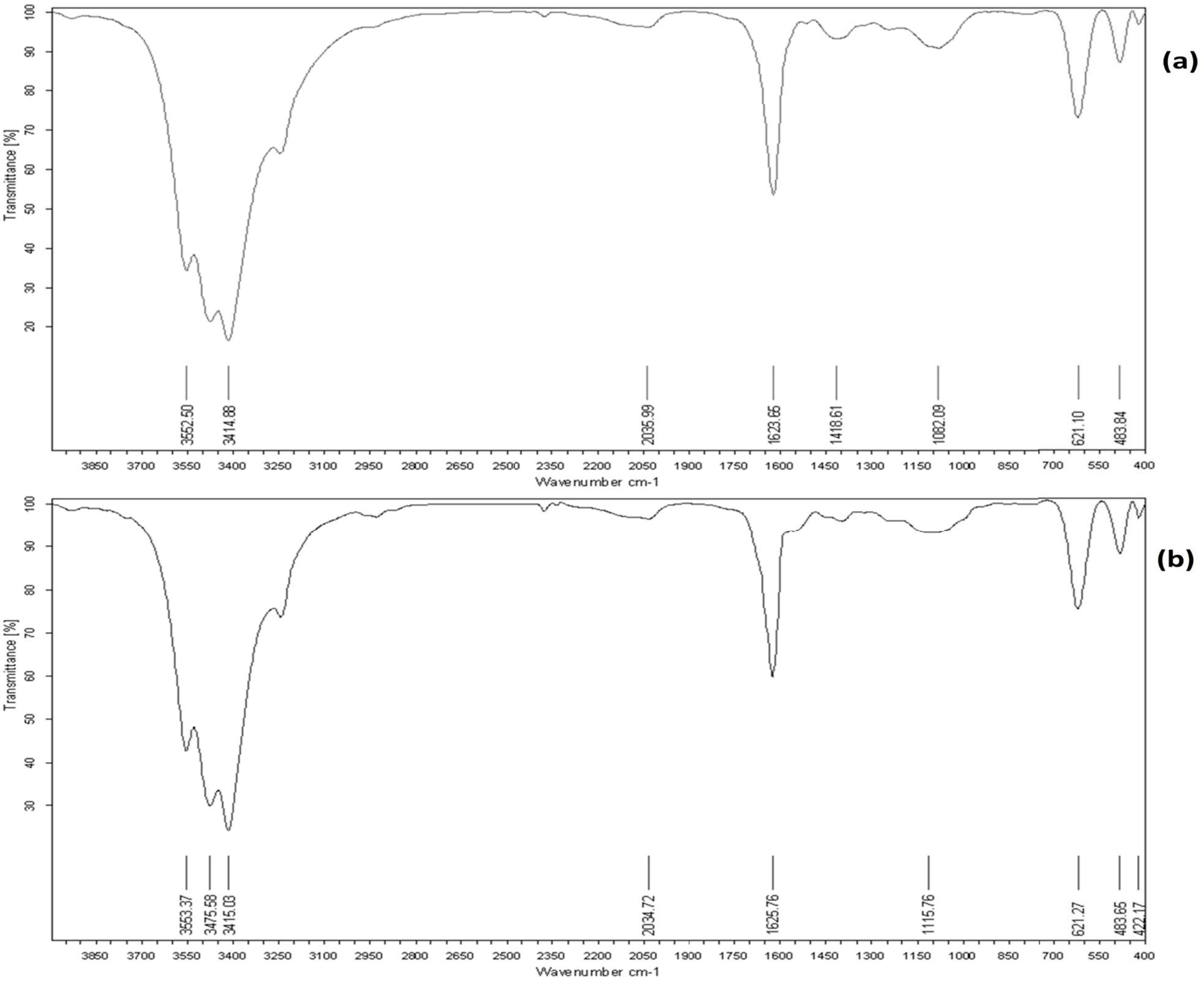

Fig. 6. FTIR spectrum of (a) *Vicia faba* and (b) *Opuntia ficus indica*.

the screening study indicated that coagulation–flocculation at a pH of 5 and by using faba bean or cactus cladodes at a concentration of 10 mg/L under a rapid-mixing speed (200 rpm) followed by a slow-mixing step at 40 rpm and without the use of flocculant, allowed for high abatement yields which could be comparable with the abatement yields obtained by the conventional coagulants. These results were confirmed by the validation test performed at the optimal parameters predicted by the experimental research methodology and which showed a removal percentage of more than 85% for both natural coagulants. However, operating the coagulation–flocculation at a pH of between 6 and 7 and by using a coagulant dose of 5 mg/L allows also for a significant reduction of the studied responses (Figs. 1–5), which allow the process to be benefic and convenient for economical consideration.

The positive influence of acid pH (5) could be due to the interaction of biomolecules present in the extract of the tested natural coagulants. The presence of some functional groups such as –COOH, OH^- ion, characterized by FTIR and chemical assays, are reported enhancing the coagulation ability following their interaction with positively charged ions, these interactions are more promoted at acidic pH [44]. The same study suggested that better coagulation activity using bio-coagulant could be obtained at neutral pH of water, which is very economical and convenient to perform the treatment a real pH, this is in agreement with the results obtained in the present study.

Coagulant dosage is one of the most influencing parameters on the coagulation activity, thus insufficient dosage or overdosing would result in poorer performance in treatment, for this reason, there is a necessity to optimize the coagulant concentration to minimize the dosing cost and sludge formation. The high coagulation activity in water contaminated by *M. aeruginosa* was achieved by using 10 mg/L of extracts. Nevertheless, smaller doses of 5 mg/L could also be used, since no significant difference between both doses in term of removal efficiency was observed (Figs. 1–5). Therefore, the positive effect achieved with natural coagulant dose of 5 and 10 mg/L could be due to the amount of active components and their structure, in relation to several factors namely the initial concentration of cyanobacterial

Table 4
Analyses of the extracts from *Vicia faba and Opuntia ficus indica*

Parameters	*Vicia faba*	*Opuntia ficus indica*
Total protein (mg/L)	312 ± 10	21 ± 5
Total phenolics content (mg eq of gallic acid/L)	766.14 ± 3.87	846.10 ± 5.83
Flavonoids content (mg eq of catechin/L)	279.28 ± 2.70	275.95 ± 3.63
Total tannins content (mg eq of tannic acid/L)	0.07 ± 0.01	0.25 ± 0.01
Total sugar (mg/L)	1,088 ± 34	1,566 ± 13
Phytic acid (mg/L)	80.67 ± 1.04	3.94 ± 0.76

cells and the pH during the coagulation–flocculation process. A similar study investigated the performance of *V. faba* to treat synthetic turbid water (stock kaolin suspension), reported that the best coagulation activity was shown with doses of 0.125 and 0.25 mL/L with a reduction of 51.5% and 54%, respectively [39]. Vishali and Karthikeyan [44], investigated the influence of different coagulant dose of *O. ficus indica* in the treatment of simulated paint industrial wastewater, authors reported that the maximum removal efficiency of 80.44% was achieved at 3 g/l00 mL to treat a liter of effluent. This was reported due to the immense amount of active components, which are responsible for coagulation activity.

Moreover, the relevance of slow-mixing step was also investigated to evaluate its effect on coagulation–flocculation activity. The results showed that the absence of slow-mixing has a negative influence on coagulation activity. The presence of slow-mixing speed is, therefore, mandatory to increase the coagulation yield by providing detention times to adequately form the desired flocs. Indeed, the kinetics of floc formation depends on the gradient velocity, while agitation increases the gradient velocity and therefore promotes flocculation by acting on the probability of microflocs meeting according to Smoluchowski's law [59].

Previous studies reported that the type of coagulant and mixing conditions govern the properties of flocs [60–62]. Onen et al. [63] demonstrated that the floc properties were influenced by the effect of the coagulant type and slow-mixing speed. However, other study had demonstrated that the improved slow-mixing speed caused the increase in the suspension stability, which leads to reduce the floc size and improve the fractal dimension [63,64].

Different rapid-mixing speeds were investigated to test its influence on the coagulation process. The results showed that the optimal rapid-mixing speed was 200 rpm. Mixing conditions during coagulation are another important factor affecting the formation and breakage of flocs. In fact, rapid-mixing allows the mixing and dissolution of reagents, homogenization of the solution, formation of polycharged species, and promotes the destabilization of coagulation phase particles.

It has been found that slow-mixing speed is related to floc characteristics: as the mixing speed increases, floc size decreases, while the floc strength, recoverability, and floc dimension improves. Lin et al. [65] observed that mixing speed influenced the floc properties, which was correlated to the coagulation mechanism involved. Additionally, many efforts have been made to verify that mixing speed should be properly matched with the type of coagulant and the coagulation mechanisms. Nan et al. [66] studied the coagulation by polyaluminum chloride (PAC) under different mixing speeds for the treatment of synthetic turbid water made by kaolin clay suspension. The authors reported that the coagulation performance and floc characteristics were slightly influenced by the mixing speed.

The effect of the flocculant addition was also tested, the results showed no significant difference with and without the addition of flocculant. These results can be explained by the time of slow-mixing prolonged up to 30 min, which favored the formation of flocs without the intervention of the flocculant.

The assessment of coagulating potential of *V. faba* seeds and *O. ficus indica* extracts were conducted in comparison with chemical coagulants (Al_2SO_4 and $FeCl_3$). Our findings confirmed that these two natural coagulants allowed for a high coagulation–flocculation activity of more than 85%. Likewise, several studies suggested the application of *V. faba* seeds and *O. ficus indica,* as natural coagulants to sanitize turbid water [17,39,44,67].

Extract of faba bean, in this study, contained higher concentration of phenolics compounds, phytic acid, and sugar and lower amount of proteins as compared to those reported in a study conducted by Kukić et al. [39] on faba bean and common bean analyzed by Kukić et al. [68]. In these two studies, the coagulation activity performance was higher. Regarding cactus cladodes extract, the amount of proteins and total phenol was lower than dried cladodes of *O. ficus indica* variety of Altixo and variety of Milpa analyzed by Bensadón et al. [69] and *O. ficus indica f. inermis* (spineless cladodes) analyzed by Ayadi et al. [70]. On the other hand, the concentrations of total sugar are higher than those reported by Ayadi et al. [70]. The difference between the concentrations found in the literature depends on the variety of the plant material, the part used, the nature of the extract analyzed, and the extraction method.

Nevertheless, Crépon et al. [71] suggested that faba bean seeds contain some constituents that can have significant anti-nutritional effects including tannins, phenolic components that are widely present in plants. Betatache et al. [17] mentioned that the majority of *O. ficus indica* juice constituents are proteins, carbohydrates, lipids, lignin, and uronic acids. According to Ndabigengesere et al. [72] and Ghebremichael et al. [73], proteins are the active components in plant extract responsible for the coagulating effect. Some authors suggest that active coagulating agents in extract from *M. oleifera* seeds are dimeric cationic proteins [72,74]. However, Sanghi et al. [34] and Okuda et al.

[75] suggested that the active components are not protein, polysaccharide, or lipid, rather some other organic polyelectrolytes. However, Özacar and Sengil [76], indicated that tannins can be a source of coagulation agents and that they can be used in water treatment. They also investigated tannins obtained from *Valonia* sp. as primary coagulant and coagulant aid, and the results demonstrated that tannins are much more effective as a coagulant adjuvant that as a primary coagulant [76].

Another study performed by Miller et al. [77], has suggested that the mucilage, specifically the galacturonic acid component and the polymers naturally present in *Opuntia* sp. may explain some of the turbidity reduction. They reported also that independently, arabinose, galactose, and rhamnose displayed no coagulation activity; however, added in combination with galacturonic acid, these sugars were able to reduce turbidity between 30% and 50%.

It was presumed that not only the proteins and polysaccharides are responsible for the coagulating effect of *V. faba* seeds and *O. ficus indica*, but other compounds such as phenols, flavonoids, tannins, and phytic acid may also contribute to the coagulation activity of investigated extracts. As such, it is likely that there are additional components of the *V. faba* and *O. ficus indica* beyond those found in the analyses of the extracts studied that could contribute to the observed coagulation activity.

In view of the preceding considerations, it is felt that *V. faba* seeds and *O. ficus indica* cladodes can be a better natural coagulants than conventional coagulants used in water treatment plants.

5. Conclusion

The design of experiments was used to study all the factors considered to influence coagulation–flocculation activity. This method is considered as economical and less costly. Thus, the effect of different parameters such as mixing speed, type and dose of coagulant, the addition of flocculant, and pH were studied to explore the potential of *V. faba* seeds and *O. ficus indica* cladodes as natural coagulants. After conducting the coagulation and flocculation test, the results show that these two natural coagulants were able to reduce the turbidity of the water, chlorophyll a, and carotenoids by up to 85% at the flowing conditions: pH 5, coagulant dose of 5 and 10 mg/L, absence of flocculant, under the presence of slow-mixing, and rapid-mixing speed of 200 rpm. Therefore, *V. faba* seeds and *O. ficus indica* cladodes were identified as an eco-friendly and as an alternative to conventional chemical coagulants to treat water contaminated with the cyanobacterium *M. aeruginosa*. NaCl extracts contain significantly several components that are predefined as responsible for the coagulation–flocculation activity, and which are analyzed by chemical assays and FTIR spectral analysis. Relaying on the previous finding, *V. faba* seeds and *O. ficus indica* cladodes could be used as natural coagulants in water system plants to reduce cyanobacterial Harmful Algal Blooms (CyanoHABs).

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