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Water Practice & Technology Vol 15 No 4
doi: 10.2166/wpt.2020.076

Eco-friendly approach for elimination of olive mill wastewaters (OMW) toxicity using cactus prickly pears juice as a coagulant

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Abstract

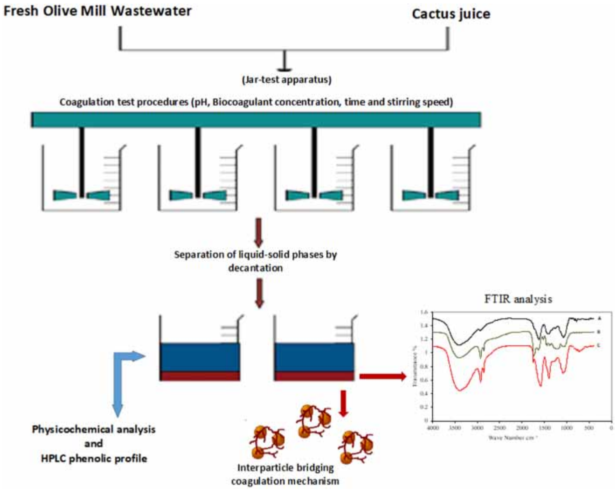
Currently, research focuses on the application of newer biocoagulant products in wastewater treatment. In this study, the performance of cactus juice to clarify Olive Mill Wastewater (OMW) was investigated by using Jar-test experimentation and assessed by physicochemical analysis of the obtained supernatant: turbidity, total suspended solids (TSS), (λ_{465}; λ_{395}) absorbance, polyphenols (pp), chemical oxygen demand (COD) and Fourier-Transform-Infrared spectroscopy (FTIR) on lyophilized sludge. A series of experiments were conducted to estimate the influence of various experimental parameters, such as the amounts of biocoagulants used, time, stirring speed, and pH. The study showed the optimal coagulation conditions were as follows: sample pH_{10}; cactus juice dose, 10% (v/v); rapid mixing time, 30 s at a speed of 150 rp/min. Under these conditions, the overall removals of 74% for COD, 93% for turbidity and 51% for polyphenols were achieved. As referring to the FTIR analysis, the cactus juice of *Opuntia* spp. operates predominantly through an adsorption interparticle bridging coagulation mechanism. These results are encouraging in the context of developing a low-cost technology and eco-friendly approach for the effective management of OMW.

Key words: adsorption-bridging, cactus juice coagulant (CJC), FTIR spectroscopy, OMW, phenolic compounds, turbidity

Highlights

- Cactus juice biocoagulant is capable of removing suspended solids from olive mill wastewater;
- the use of cactus juice in coagulation/flocculation treatment is a low-cost technology.
- the use of cactus juice in coagulation/flocculation treatment is an eco-friendly approach for effective management of OMW.
- The accessibility to the raw material is very easy and cultivated at a large scale in Morocco, this fact could make the process very rentable.
- Cactus juice does not leave residual toxic elements that can cause secondary contamination; it should be biodegradable and presumed to be safe for human health.

Graphical Abstract



INTRODUCTION

Coagulation and flocculation are processes by which small particles are connected forming large enough flocs to be separated from solution in a reasonable amount of time. They constitute the backbone processes in most water and advanced wastewater treatment plants, and are frequently applied processes in the primary purification of industrial wastewater (and in some cases in secondary and tertiary treatment) (Jiang & Graham 1998; Manu 2007). Aluminum-based salts, iron-based salts (ferric chloride) or organic polymers are the most common chemicals used as water treatment coagulants. Unfortunately, they have a major drawback when used in drinking water treatment since the toxicity of the chemicals must be taken into account and be checked. Recently, there has been increasing interest in the use of environmentally friendly technologies, using natural organic coagulants that can be extracted from microorganisms, animal or plant tissues (Ganjidoust *et al.* 1997). They replace with success synthetic flocculants or inorganic salts of Al or Fe, such as $AlCl_3$, $Al_2(SO_4)_3$, $FeCl_3$, $Fe_2(SO_4)_3$, etc., in water treatment. The use of natural coagulants of vegetable origin to clarify turbid raw waters is not a new idea. Natural coagulants were used in water treatment before the advent of synthetic chemicals like aluminum and ferric salts (Ndabigengesere *et al.* 1995; Cheng & Chi 2002). It has been reported that communities in tropical rural areas have used these for the domestic household for centuries in traditional water treatment (Fuglie 2001). A method known in some countries for clarifying drinking water uses cactus mucilage as an agent and has been used for many years by small farmers in Chile. Some authors have investigated the capacity of natural plant tissue coagulants in water and wastewater treatment. Many of them have researched about *Moringa oleifera*, indigenous to Sudan (Ndabigengesere *et al.* 1995; Ghebremichael *et al.* 2005; Krishna Prasad 2009), others about Nirmali seed (Adinolfi *et al.* 1994; Jayaram *et al.* 2009; Sarawgi *et al.* 2009), seed gum and gum-g-polyacrylamide of *Cassia javahikai* in textile wastewater treatment (Sanghi *et al.* 2006) and different leguminous species (Šciban *et al.* 2005). Our current study is one of many studies in which *Opuntia ficus indica* is used as a biocoagulant to remove turbidity and total suspended solids from raw olive mill wastewater (Table 1). Zhang *et al.* (2006) examined the use of cactus (Cactaceous *opuntia*) in the treatment of seawater, urban wastewater and a potable water source. He found that the turbidity removal efficiency could reach 94%, the optimum pH was about 10 and temperature had a slight influence on the coagulation effect. Miller *et al.* (2008) discussed the effect of parts and forms of the *Opuntia* spp. pad on the presence or absence of coagulation activity in a model of drinking water with kaolin. They found that the maceration or heating above ~100 °C has a losing efficacy effect

Table 1 | Effect of plant extract on turbidity removal of different sampling water and the suggested mechanism hypothesis

Plant extract	Party sought	Sampling treated	Mechanism hypothesis	Optimum condition parameters	Removal turbidity (%)	Reference
Cactus *latifaria*		Synthetic water with kaolin	Not suggested	• Dosage 10 mg·l^{-1} • pH = nd	Up to 90%	Diaz *et al.* (1999)
Cactus *Opuntia ficus-indica*	Juice of cladode (CJC)	Olive mill wastewater (OMW)	Adsorption-bridging	• Dosage 10% • pH = 10	93%	Neffa *et al.* (2012)
Opuntia spp	Fresh pad Dry pad	Model drinking water with kaolin	Bridging-coagulation	• Dosage: 1. Low turbidity 5–15 mg 2. Medium turbidity 15–35 mg 3. High turbidity 35–55 mg • pH = 8–10	92% to 99%	Miller *et al.* (2008)
Cactus *Opuntia spp.*	Dry solids 0.45–1.25 μm	Synthetic water with kaolin Sewage water seawater	Not suggested	• Dosage: 50 mg 60 mg 60 mg • pH = 10	94% 84% Up to 98%	Zhang *et al.* (2006)
Cactus *Opuntia spp.*	Pad and inner skin 53–106 μm	Estuarine water River water	Not suggested	• Dosage 13 mg·l^{-1} • pH = nd	98.2% 69.7%	Yin *et al.* (2007)

of the coagulation agent. The same authors pointed out that the hypothesis of the coagulation mechanism for *Opuntia* spp. is explained by adsorption and bridging.

The industry of olive oil extraction, an agro-industrial activity of vital economic significance to many Mediterranean countries, is unfortunately associated with the generation of large amounts of black olive mill wastewaters (OMW) and solid wastes, whose management, treatment and safe disposal raise serious environmental concerns. OMW has significant polluting properties, especially due to the high concentration of organic content and total suspended solids (TSSs) loads as well as acidic pH and the presence of toxic pollutants such as polyphenols. In Morocco, olive oil production and the subsequent wastes are widespread in small locations, resulting in intensive pollution problems. OMW is discharged into nature without any prior treatment, which causes acute deterioration of the quality of the receiving media including rivers, groundwater and agricultural soils (Boukhoubza *et al.* 2008). To overcome these difficulties, an innovative physicochemical eco-friendly approach, using cactus juice as a coagulant (CJC), can be easily introduced to produce a dilute solution on organic matter and total suspended solids (TSSs). The reasons for the choice of CJC in wastewater treatment using coagulation/flocculation processes are mainly:

- The accessibility to the raw material is very easy as it is cultivated at a large scale in Morocco and in the areas where the production of OMW is concentrated. This fact could make the process very rentable;
- Cactus juice has the advantage of being a non-toxic material, low price, non-corrosive and easy to handle;
- The removal of TSS from OMW could lead to the formation of organic sludge which may be reused, saving money on sludge disposal;

- Cactus juice does not leave residual toxic elements that can cause secondary contamination; this coagulant should be biodegradable and is presumed to be safe for human health.

The tightening of legislation and strengthening of safety requirements encourage the olive oil extraction industries to improve continuously the quality of their effluents. The establishment of a treatment system in these units corresponds to a regulatory requirement and an environmental need, whatever it is conditioned by several regulatory, environmental, social but above all economic constraints. The large majority of primary treatment systems are based on the coagulation/flocculation process. Unfortunately, this treatment remains relatively polluting, because it uses products based on metal salts (mainly aluminum and iron) and synthetic polymers (polyacrylamides, etc.). We propose to these industries a sustainable strategy based on eco-friendly practices. The proposed treatments are a green process that respects the environment and allows the recovery and reuse of these effluents after treatment. The most important thing in using CJC is the accessibility of the raw material at low cost and the ease of handling safely. To the best of our knowledge, there are very few works that used cactus juice as a coagulant in the OMW treatment and offer it as a practical solution to the olive oil extraction units. In this study, coagulation/flocculation performance of cactus juice as coagulant was investigated in terms of turbidity, TSS, chemical oxygen demand (COD), (λ_{465}; λ_{395}) absorbance and total polyphenols removal from OMW, using jar test to assess the optimal system variables (mucilage dose and pH). Furthermore, a detailed investigation supported by FTIR spectra analysis was also conducted to understand the mechanism of coagulation.

EXPERIMENTAL AND ANALYTICAL

OMW used

Fresh olive mill wastewater was supplied by a semi-automatic phase olive processing plant located in the province of Tadla-Azilal (Morocco). This OMW is collected from immediately triturated olives and stored at 4 °C.

Cactus juice preparation

A 5 kg of fresh prickly pear cladodes (*Opuntia ficus indica*) were collected from its natural environment, near the city of Marrakech, Morocco. The harvested cladodes were washed by tap water. The peeled cladodes were cut off into big cubes and the skin was peeled from the pad, then crushed in a blender (Moulinex) with relatives knifes type (Bleno DAE201). The resulting extract was filtrated by a filter paper then centrifuged at 1,500 r.p.m. for 20 min at 10 °C. The supernatant was then transferred to a clean flask, sealed by a parafilm and then left in the refrigerator (4 °C) till used.

Coagulation test procedures

The experiment was performed using (Phipps & Bird) jar-test apparatus equipped with a six-place paddle stirrer, which can be simulated to a clarifier under mixing and settling accurate conditions. Jars (beakers) with different treatment programs and the same coagulant at different dosages are run side by side, and the results compared to an untreated jar. In each beaker of 1 L capacity, we filled 200 mL of crude OMW. After that, we placed the filled jars on the gang stirrer, with the paddles positioned identically in each beaker. In this study, the pH has been varied in the range 2.0–10.0 in an attempt to investigate the influence of this parameter on OMW treatment. The pH value of each jar test was adjusted to the desired value by using either chlorydric acid or sodium hydroxide (10 M) within the range of 2.0–10.0. At the optimum pH, different CJC doses were tested (2% to 12% (v/v)).

Unless specified otherwise, the crude OMW sample was agitated at 150 rpm for 3 min (rapid mixing). The mixing speed was reduced to 30 rpm for 30 min. The contents of each beaker were then allowed to settle for 60 min. The supernatant was analyzed at the end of each essay.

Materials and analytical procedures

Physicochemical analyses

The physicochemical analyses were performed on cactus juice coagulant and on raw and treated OMW studied according to standardized methods. The chemical oxygen demand (COD) was determined by a colorimetric method (APHA 1992). After digestion at 150 °C under sulfuric acid and potassium dichromate, in the presence of mercury sulfate and silver sulfate. the absorbance of the samples is measured by a spectrophotometer (UNICAM Model 8625 UV/VIS spectrometer) at 620 nm. Biochemical oxygen demand (BOD_5) was assessed by measuring the oxygen uptake in a sample over 5 days at 20 °C, in the dark, using the BOD Track apparatus. The sample was diluted, mixed with domestic wastewater and its pH was adjusted to 7.00. As dissolved oxygen was consumed, air pressure in the bottle dropped and pressure changes were continuously recorded (AFNOR, T 90-103). Total suspended solids (TSS) was determined after filtering a sample through a GF/C filter and drying the retained residue at 105 °C for 4 h (AFNOR T 90-105). The discoloration of OMW was assessed by the difference in absorbance, read at 395 and 465 nm between OMW before and after treatment by cactus juice (Kissi *et al.* 2001). The visible absorbance at 465 nm can also be used as it's well correlated with a great number of aromatic rings or long conjugated bonds in aromatic structure (He *et al.* 2010).

Kjeldahl Nitrogen (TKN-N) was determined by the Kjeldahl digestion and distillation method (AFNOR T90-110). Ammonia nitrogen (NH_4^+) was determined by indophenols colorimetric method according to AFNOR 1983. Nitrite (NO_2^-) was determined by coupling diazotation followed by a colorimetric method according to the AFNOR method. Nitrate (NO_3^-) was determined like nitrite, after its reduction by the passage in a cadmium-copper column (Rodier 1984). The phosphorus content (expressed as phosphate equivalents) was determined by the ascorbic acid colorimetric method according to the AFNOR method. Chloride (Cl^-) was determined by silver nitrate titration method according to the AFNOR method (AFNOR T90-014). Reducing sugars were determined at 490 nm by a colorimetric method according to Dubois *et al.* (1956). Simple sugars, oligosaccharides, polysaccharides, and their derivatives, give an orange-yellow color when treated with phenol and concentrated sulfuric acid (Dubois *et al.* 1956). K^+, Na^+, and Ca^{2+} present in OMW were analyzed with a flame photometry method (JENWAY, PFP7).

Ion chromatography apparatus

For cations determination in mucilage, the Dionex Model ICS 1100 ion chromatography apparatus was used, with an IonPac CS12 guard column and an IonPac CS 12 separator column, a cation self-regenerating suppressor (4 mm) and a Dionex conductivity detector CSRS 300. Similarly, for the determination of anions, an IonPac AS 23 guard column, an IonPac AG 23 separator, an anion suppressor (4 mm) ASRS 300, a spectrophotometric variable-wavelength detector was used. Samples were injected automatically using a 20 μl loop injection valve. The analysis was performed with a flow rate set at 1 mL/min. Eluent A (20 mM of Methane sulfonyl acid) and eluent B (4.5 mM Na_2CO_3; 0.8 mM $HNaCO_3$) were used for cations and anions determination respectively during the procedure.

Infrared (FTIR) spectroscopy characterization

The cactus juice of *Opuntia* spp. and obtained sludge after treatment of OMW were collected and freeze-dried (Christ ALPHA 2-4 LD plus). For infrared analysis, 1 mg of lyophilized samples was mixed with 250 mg KBr (FTIR grade, Merck), and 1 mm a pellet was prepared using a hydraulic press. Transmission Fourier-Transform InfraRed (FTIR) spectroscopy was conducted with a Bruker Vertex spectrophotometer. The spectra were recorded in the 4,000–400 cm^{-1} range with 32 scans collected at 4 cm^{-1} resolutions.

Liquid-liquid extraction of polyphenols and HPLC analyze

The liquid-liquid extraction of phenolic fraction has been carried out according to the method described by Macheix *et al.* (1990) and the determination of total phenols was estimated by using the Folin–Ciocalteu reagent (Flouri *et al.* 1996), the absorbance was read at 760 nm and converted into equivalent Tyrosol (g/l). The phenolic extract was analyzed by high-performance liquid chromatography (HPLC) on a JASCO HPLC system, consisting of a-waters Delta Prep 3000 model quaternary solvent pump, equipped with a vacuum degasser (Knauer D-14163 Berlin, Germany). A reversed-phase Lichrosphere C–18 (4 × 250 mm i.d 5 μm) column, a JASCO UV intelligent detector (UV-975) operating at 280 nm and Galaxy software for data acquisition were used. Using an isocratic mobile phase (90% H_2O (containing 1% acetic acid)/10% Acetonitrile), the flow rate was 0.7 $mL{\cdot}min^{-1}$ and the injection volume was 20 μL. Finally, the phenolic extract was filtered through a 0.45 μm nylon filter, and 20 μL was injected into the chromatograph. The identification of phenolic compounds, for which standards were available, was carried out by comparison of their retention time and their UV–visible spectra with those of the standards in the same conditions, while the presence of other compounds was confirmed by comparison of UV–visible spectra to those of standards of the same polyphenolic family and bibliographic data.

RESULTS AND DISCUSSION

OMW physicochemical characterization

Crude OMW is dark brown colored and foul-smelling. Table 2 shows the main physicochemical characteristics of the average compositions of the OMW. The pH of the wastewater is around 4.7 with a medium conductivity. The value of biochemical oxygen demand and chemical oxygen demand was very high, proving a high organic load of OMW, and must be treated before discharge into the environment. Also, the concentration of total phenolic compounds exceeds 8 g of $Tyrosol{\cdot}l^{-1}$ with a high ratio of COD/BOD_5 (4.8), which makes OMW difficult to treat biologically (Sayadi *et al.* 2000; Tezcan-Un *et al.* 2006). OMW contains also large amounts of suspended solids and total sugars which indicates the state of OMW freshness. As can be seen in Table 2, the inorganic cations are mainly K^+, Na^+, Ca^{2+}, Mg^{2+} and total Kjeldahl nitrogen (TKN) concentration were very high. HPLC analyses showed that the most phenolics compounds were monomeric, with a predominance of Hydroxytyrosol, Tyrosol, Catechin, caffeic acid and others (Figure 1).

Impact of cactus juice coagulant dose on treatment performance

Variation in TSS and turbidity as a function of CJC concentration

It should be noted that biocoagulation/flocculation by CJC produces larger and better floc quality. These flocs sediment more quickly. Figure 2 shows the removal of turbidity and TSS as a function

Table 2 | Physicochemical composition of raw OMW (mean (3) ± standard deviation)

Parameters	Unity	OMW value
pH		4.70 ± 0.05
Turbidity	NTU	8,490
Absorbance	$\lambda = 395$ nm $\lambda = 465$ nm	157.7 ± 7.0 69.3 ± 5.0
Total suspended solids	$g{\cdot}l^{-1}$	14.72
Chemical oxygen demand	$g\ O_2.l^{-1}$	294 ± 6
Biological oxygen demand	$g\ O_2.l^{-1}$	61.15
COD/BOD		4.8
Electric conductivity	$mS{\cdot}cm^{-1}$	15.00 ± 0.03
Total polyphenols	g of $Tyrosol{\cdot}l^{-1}$	8.27 ± 0.50
TKN–N	$g{\cdot}l^{-1}$	1.9 ± 0.2
NH_4^+–N	$mg{\cdot}l^{-1}$	14.52 ± 0.70
NO_3^-–N	$mg{\cdot}l^{-1}$	135.33 ± 1.10
NO_2^-	$mg{\cdot}l^{-1}$	66.95 ± 0.40
PO_4^{3-}	$mg{\cdot}l^{-1}$	173.4 ± 2
Total P	$mg{\cdot}l^{-1}$	356.4 ± 1.5
K^+	$g{\cdot}l^{-1}$	8.64 ± 0,01
Ca^{2+}	$g{\cdot}l^{-1}$	1.20 ± 0,002
Na^+	$g{\cdot}l^{-1}$	0.50 ± 0,002
$[Cl^-]$	$mg{\cdot}l^{-1}$	2,840 ± 3.1
MS	$g{\cdot}l^{-1}$	102.49
Total sugar	$g{\cdot}l^{-1}$	29.50 ± 0.05

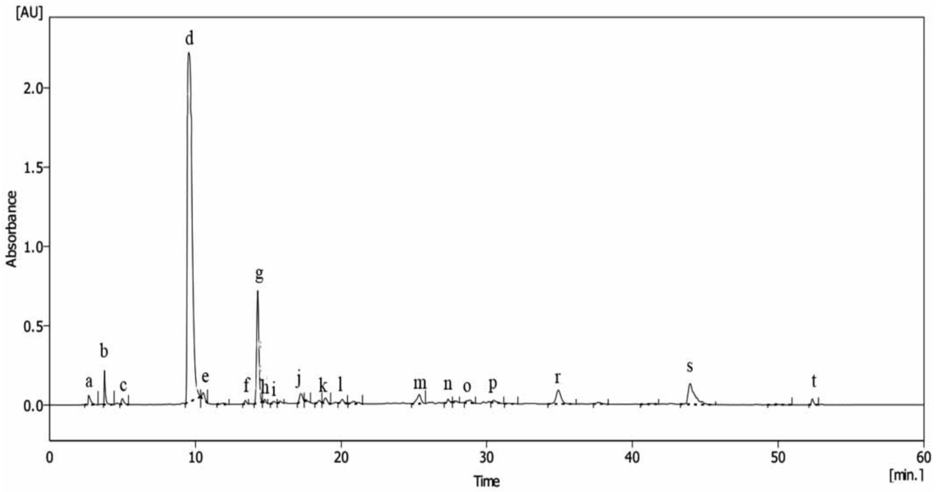


Figure 1 | HPLC-Rp-DAD analysis of polyphenols from OMW at pH_2. (a) Syringic acid; (b) gallic acid; (d): Hydroxytyrosol; (e): Hydroxybenzoic acid; (g): Tyrosol; (h): Catechin; (j): Vanillic acid; (k): caffeic acid; (m): para-coumaric acid derivative; (r): ferulic acid derivative; (s): Oleuropein; (t): Quercetin. Peaks not identified: c, f, i, l, o, p, u.

of the concentration of cactus juice coagulant at the initial pH of raw OMW (4.82). The percentage of abatement of these parameters increases with increasing dose. For doses greater than 2%, the removal percentages of TSS and turbidity increase. After, at 10% (v/v) addition of CJC, the turbidity and TSS reach maximum values of 64 and 67%, respectively. The removal percentages for TSS and turbidity decrease when the dose of coagulant goes from 10 to 12%. These findings indicate that the optimum dose is around 10%.

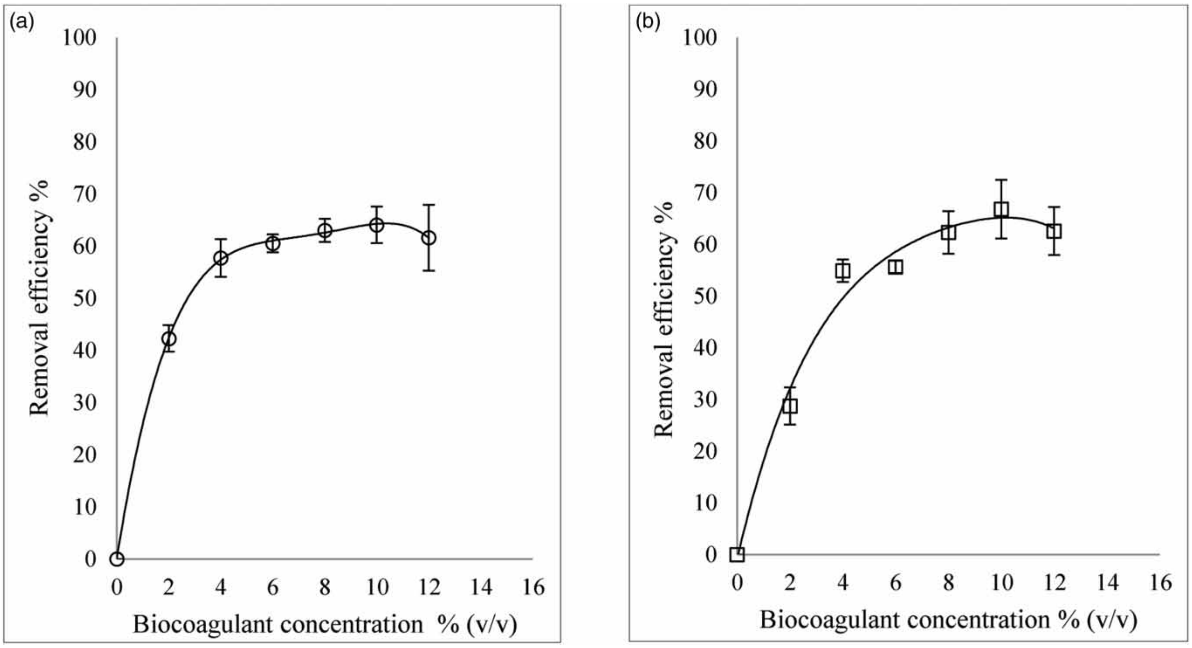


Figure 2 | Removal of turbidity (a) and TSS (b) after pretreatment and application of increasing doses ranging from 2% to 12% of CJC on raw OMW (pH = 4.82).

A high dose of the biocoagulant was required to obtain significant removal of TSS and turbidity, attributed to a large amount of organic material present in the OMW (Zayas *et al.* 2007). Around the dose of 10%, it seems that the solution has gone through the point of net electrical charge and the coagulation was optimal. These first observations let us suggest that polysaccharides species contributed to the most effective particle removal, which was due to its higher charge neutralization ability. The same observation was also reported in the works of Pinotti *et al.* (1997) and Meyssami & Kasaeian (2005) when they used chitosan as biocoagulant. This indicated that charge neutralization played an important role in the coagulation mechanism. Furthermore, according to the work reported by Miller *et al.* (2008) the use of increasing doses of *Opuntia* spp. does not alter zeta potential.

Removal of polyphenols and COD as a function of CJC concentration

The polyphenols (pp) and COD removal by CJC in a range of 2–12% were studied through standardized jar testing procedures and the results are shown in Figure 3. The optimum concentration of CJC was 10%, which yielded an optimum pp and COD removal efficiency of 31.5 and 74%, respectively. It was also observed that pp and COD removal efficiency varied slightly after 4% and this abatement remained unchanged when the biocoagulant concentration increased to 12%.

The reduction of organic matter and phenolic fraction mainly occurs through a mechanism bridging-coagulation mechanism where particles do not directly contact one another but are bound to a polymer-like material that originates from the cactus species. Miller *et al.* (2008) reported that galacturonic acid is possibly the active agent that affords the coagulation capability of *Opuntia* spp. Similar mucilage from *M. oleifera* and *P. psyllium* extracts is reported to contain carbohydrates such as d-xylose, l-arabinose, and d-galacturonic acid can remove colors and turbidity (Okuda *et al.* 2001; Mishra & Bajpai 2005).

Effect of time and stirring speed on COD and turbidity removal efficiencies

The agitation of the mixture promotes the formation of the flocs in the coagulation/flocculation process. It starts with a rapid homogenization of the effluent/biocoagulant mixture followed by slow stirring. The results show that the time and speed of this homogenization has no significant effect

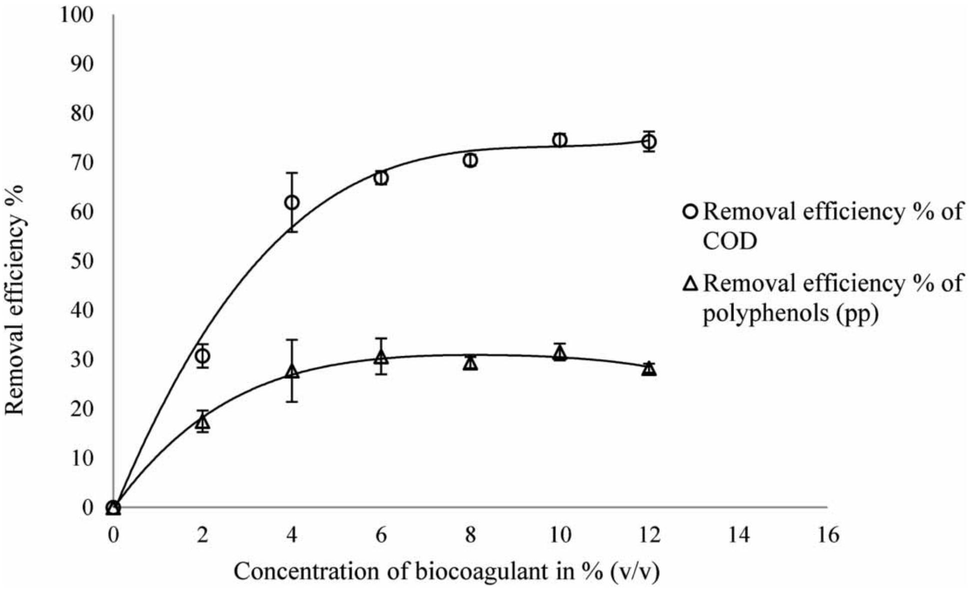


Figure 3 | Removal of polyphenols (pp) and COD at various concentrations of CJC at raw OMW (pH = 4.82).

on the reduction of COD and turbidity by the biocoagulant (Figures 4 and 5). According to the results obtained, a homogenization time of 30 seconds at a speed of 150 rp/min is enough to obtain a good reduction of organic loads and turbid particles.

Effect of initial pH on coagulation performance

In the following tests, rapid stirring was estimated at 150 rpm/min for 30 seconds, slow stirring was estimated at 30 rpm/min for 30 min, followed by a settling time of 30 minutes. Under the conditions described above, the optimal dose of 10% was applied. The initial pH is one of the important factors affecting the performance of the biocoagulation process. In this study, the pH of the effluent was varied in the range 2.0–10.0 in an attempt to investigate the influence of this parameter on the treatment of OMW.

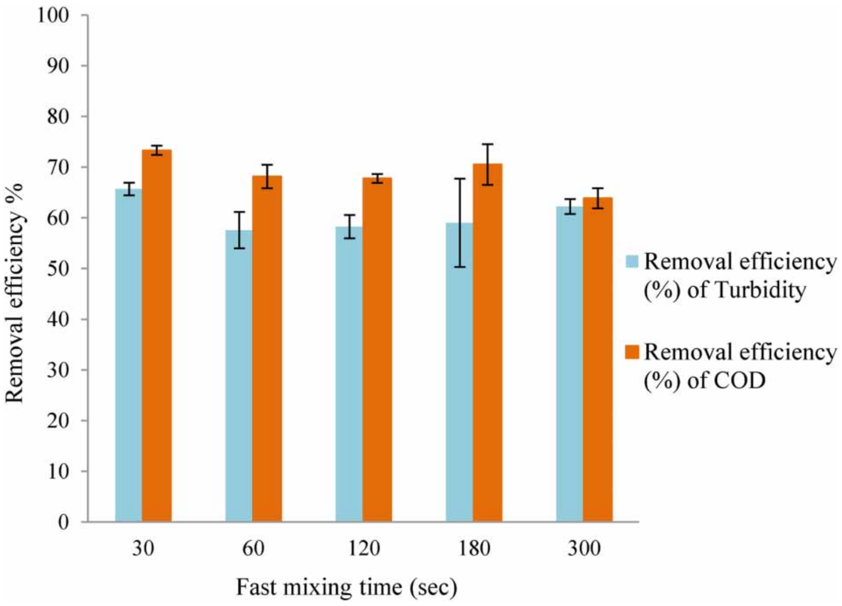


Figure 4 | Removal of turbidity and COD after pretreatment, according to various fast mixing time. Optimum concentration of CJC is 10% applied on raw OMW (pH = 4.82).

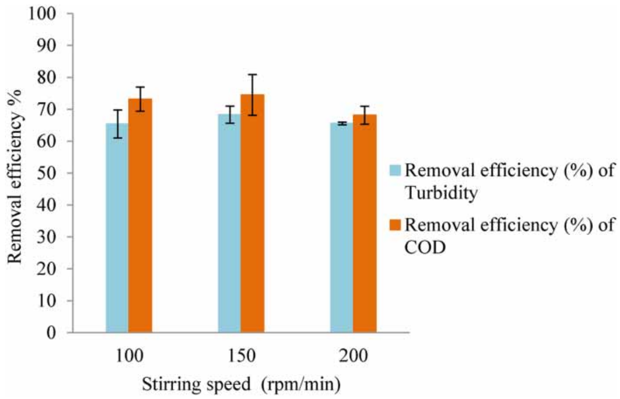


Figure 5 | Removal of turbidity and COD after pretreatment, according to various stirring speeds. Optimum concentration of CJC is 10% applied on raw OMW (pH = 4.82).

Table 3 shows the variation of initial OMW pH after coagulation in every sample. An insignificant decrease in the initial pH of OMW samples occurs after coagulation, which may be due to the high acidity of the cactus juice solution, which balanced the hydroxide ions in the OMW. This result is in agreement with Olsen 1987, who showed that the cactus coagulant does not affect the pH of OMW after pretreatment. Removal efficiencies of turbidity, TSS and dark color expressed by two wavelengths (λ_{465}; λ_{395}) as a function of initial pH were presented in Figure 6. It can be seen that removal turbidity, TSS and color (λ_{465}; λ_{395}) absorbance is significantly affected and coagulation efficiency depends on the initial pH of OMW. Biocoagulation/flocculation activity of *Opuntia ficus indica* is greatest in alkaline conditions and best removal results were observed at pH 10.0. Maximum turbidity, TSS and color (λ_{465}; λ_{395}) absorbance removal efficiencies of CJC were respectively around 93%, 89, 75 and 68% in this pH region. This instance was in good agreement with the previous finding (Zhang *et al.* 2006).

Table 3 | Variation of initial pH of OMW after pretreatment

Initial pH	Final pH
2.12	2.03
3.01	2.93
4	3.98
Crude OMW (4.82)	4.77
6	6.22
7	7.14
8.02	7.47
9	8.3
10	9.29

Figure 7 shows the effect of initial pH on removals of polyphenols (pp) and COD. Generally, the pp removal showed an upward at higher pH and reached 51% at pH 10.0. However, the removal efficiency of COD is invariable in all pH and ranged between 72.5 and 74%. The effect of pH solution on the coagulation/flocculation process with cactus *Opuntia* is rather complicated and more difficult to elucidate due to the unclear nature of its coagulation/flocculation agents. The high coagulation capability of *Opuntia* is most likely attributed to the presence of mucilage, which is a viscous and complex carbohydrate stored in cactus inner pads. The HPLC analysis showed that the major sugars present in cactus mucilage are galactose (40.81%) and arabinose (30.60%). Other minor sugars such as xylose, rhamnose, and glucose at low contents are also detected (Habibi *et al.* 2004; Espino-Diaz *et al.* 2010; Petera *et al.* 2015). It is clear that mucilage is not a single biopolymer like chitin, chitosan, or alginate whose functions are well known but it

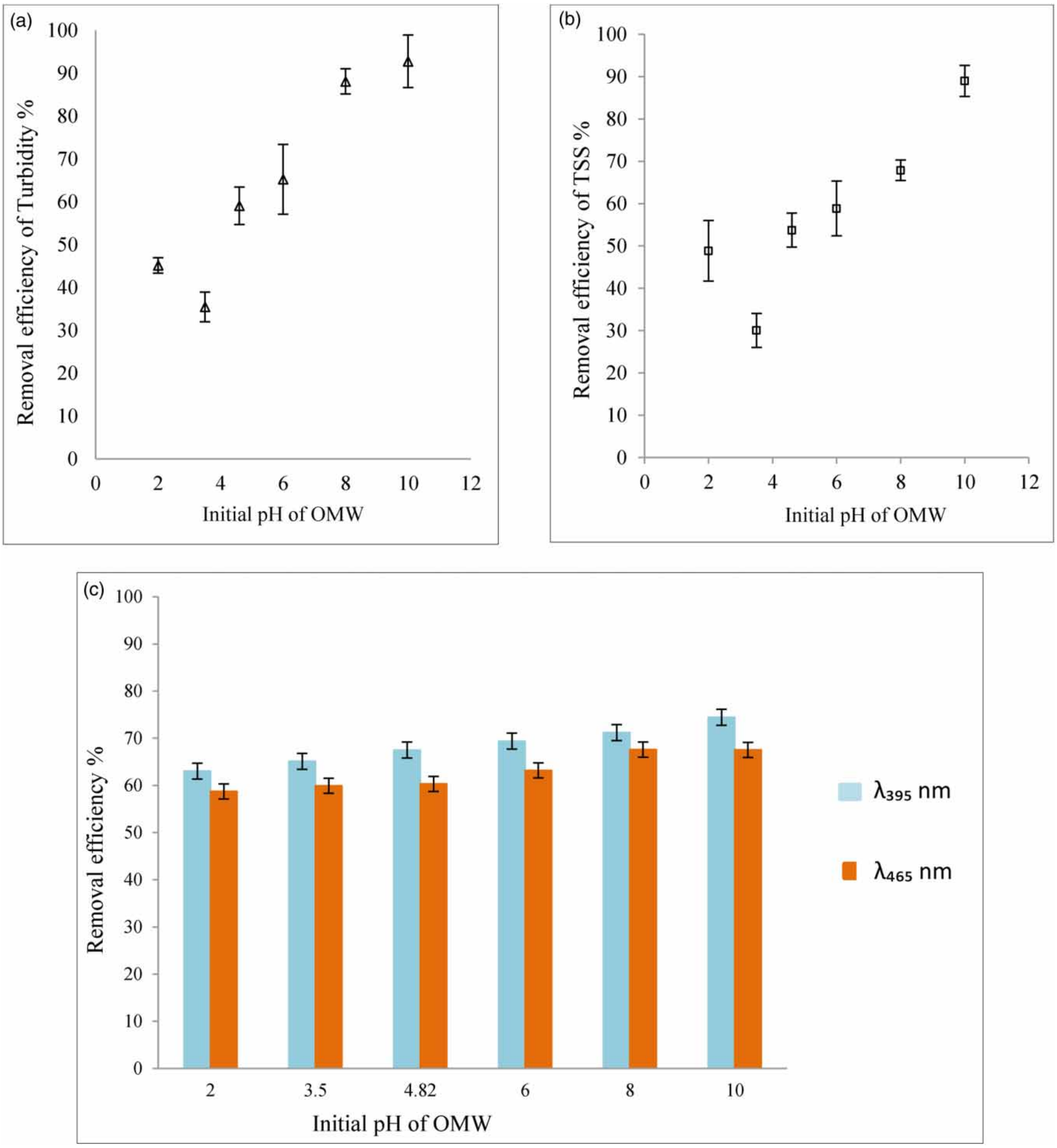


Figure 6 | Removal of turbidity (a), TSS (b), and dark color (c) expressed by two wavelengths (λ_{395}, λ_{465}) after pretreatment. The optimum concentration of CJC is 10% applied to OMW with different initial pH.

may be composed of many different polymeric units, and therefore is likely to show multiple functionalities. Miller *et al.* (2008) worked with model turbid water containing background electrolytes. Under these conditions, *Opuntia* spp. operated with greater than 98% turbidity removal between pH 8 and 10. Similarly, Zhang *et al.* (2006) have reported that *Opuntia* spp. is most effective at pH_{10} and less effective at pH6.

Discussion of possible mechanisms of biocoagulation/flocculation by CJC

Physicochemical *opuntia f. indica* properties

The prickly pear cactus (*Opuntia* spp.) represents the major branches of the *Opuntia* cactus. The mucilage of *O. f. indica*, an amorphous polysaccharide, is a natural polysaccharide (complex

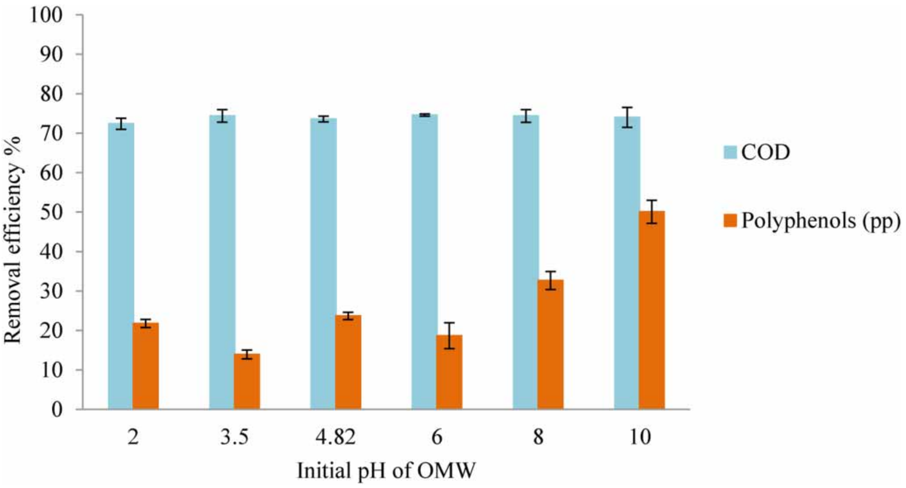


Figure 7 | Removal of COD and polyphenols (pp) after pretreatment. The optimum concentration of CJC is 10% applied to OMW with different initial pH.

carbohydrates) composed of varying proportions of L-arabinose, D-galactose, L-rhamnose, and D-xylose, as well as galacturonic acid in different proportions (Trachtenberg & Mayer 1981; Goycoolea & Cardenas 2004; Matsuhiro *et al.* 2006; Leon-Martinez *et al.* 2011). In this study, it was determined that cactus juice contained a middle amount of COD 8 g O_2/100 g of prickly pads mucilage with an acidic pH (Table 4). For mineral analysis, our results showed that the mucilage of prickly pear is rich in potassium, magnesium, and chloride; however, they contained a low amount of lithium and fluoride, and relatively low amounts of PO_4^{3-}, Na^+ and Ca^{2+} (Table 4).

Table 4 | Physicochemical parameters and mineral composition in anions and cations determined by ion chromatography (IC) on 100 g prickly pads mucilage (*Opuntia Ficus Indica*)

Physicochemical parameters	**pH**	**Chemical oxygen demand (g d'O_2/100 g)**	**Conductivity (mS·cm^{-1})**	**MS (g/100 g)**	**TKN–N (mg/100 g)**	**Total phosphorus (mg/100 g)**
Values	4,05	8 ±0,79	12,5 ± 0,02	12,88 ± 0,9	12,5 ± 0,05	26,2 ± 0,4
Anions analyses (mg/100 g)	$\mathbf{F^-}$	$\mathbf{SO_4^{2-}}$	$\mathbf{Cl^-}$	$\mathbf{PO_4^{3-}}$	$\mathbf{NO_3^-}$–N	$\mathbf{NO_2^-}$–N
	0,174	85,2	150,24	12,97	3,24	6,16
Cations analyses (mg/100 g)	$\mathbf{K^+}$	$\mathbf{Na^+}$	$\mathbf{NH_4^+}$–N	$\mathbf{Li^+}$	$\mathbf{Ca^{2+}}$	$\mathbf{Mg^{2+}}$
	170,87	29,34	3,92	0,0137	9,63	92,13

(Mean ($n = 3$) ± Standard deviation).

The infrared (IR) spectrum of the cactus juice

The FTIR spectra of lyophilized cactus juice is shown in Figure 8(a). The spectrum showed high absorbance at wave numbers characteristic of polysaccharides (1,000–1,150 cm^{-1}, 1,400–1,550 cm^{-1}, 2,800–2,900 cm^{-1}, and 3,100–3,500 cm^{-1}): a broad stretching intense characteristic peak at around 3,435 and 3,428 cm^{-1} for the hydroxyl group and two weak C–H stretching bands at 2,926 and 2,850 cm^{-1} (Manuel *et al.* 1999; Santhiya *et al.* 2002). The 1,720 cm^{-1} band is assigned to C = O stretching vibrations of the non-ionized carboxylic groups. There is a clear presence of two bands associated with symmetric and asymmetric COO^- stretching vibrations centered approximately 1,600–1,650 and 1,400–1,450 cm^{-1}, respectively (Manrique & Lajolo 2002; Zhao *et al.* 2007). The absorbance of polysaccharides in the range 950–1,200 cm^{-1} appeared where the C–O–C and C–O–H link band positions were found (Kacura kova *et al.* 2000; Zhao *et al.* 2007).

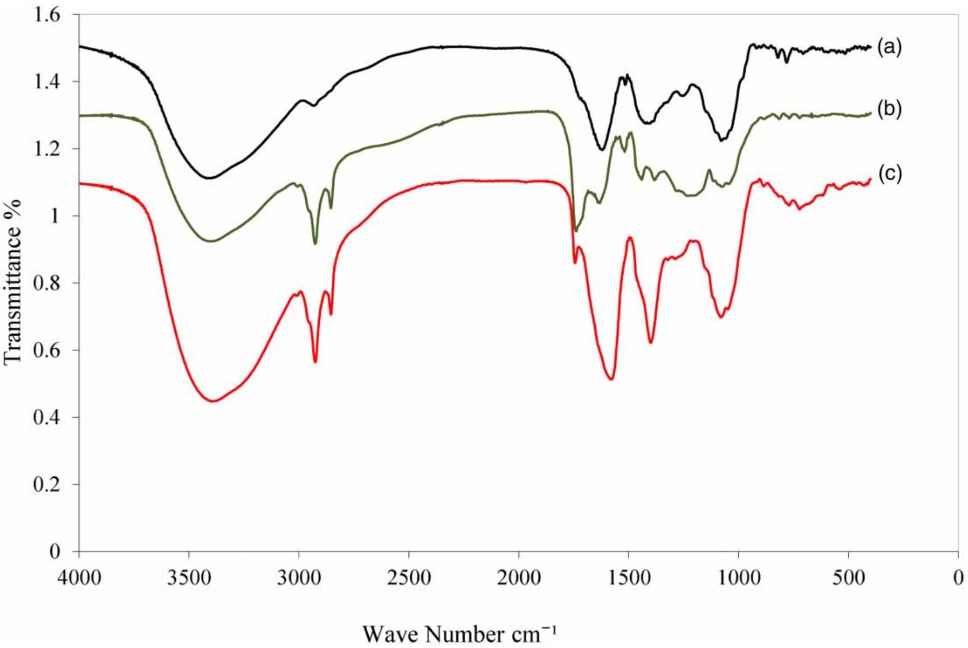


Figure 8 | Absorbance spectra of FTIR spectroscopy in the 4,000–400 cm^{-1} region as a function of (a) lyophilized cactus juice rich in polysaccharides (b) lyophilized OMW sludge after pretreatment at (pH$_2$) and (c) lyophilized OMW sludge after pretreatment at (pH$_{10}$).

Basic mechanisms for the destabilization of colloidal particles and organic matter by CJC

The IR-TF spectrum shows that the composition of the polysaccharide provides on this mucilage a macromolecular structure carrying many chemical groups. The diversity of these groups on the biopolymers of the polysaccharide leads to a variety of functions and possible mechanisms for the coagulation/flocculation process. The mucilage of cactus can be qualified as a polyelectrolyte, which has an intrinsic capacity to interact with colloidal particles and dissolved organic matter at a generally negative charge. Under the usual conditions of pH (5–10), all the particles carry a negative charge and are often colloids resistant to aggregation. The mucilage of cactus used in this experience essentially interacts by a double mechanism: adsorption and interparticle bridging coagulation mechanism (Figure 9). Miller *et al.* (2008) and Jadhav & Mahajan (2014) showed that the predominant mechanism in coagulation was adsorption and bridging in the treatment of model turbid water using *Opuntia* spp. A similar mechanism was postulated by Fedala *et al.* (2015) and Nharingo *et al.* (2015) during the coagulation process applied to raw surface water from a dam and Pb (II) removal from wastewaters respectively, using *Opuntia ficus indica* biomass.

Fourier transformation infrared (FT-IR) spectroscopy study of binding sites

An analysis comparison between FTIR spectra of lyophilized sludge obtained after pretreatment of OMW at pH$_2$ and pH$_{10}$ and the FTIR spectrum of lyophilized cactus juice was performed. This analysis allowed us to observe which peaks shifted after pretreatment and to elucidate some mechanism of cactus coagulation/flocculation. The results (Figure 8 and Table 5) show that all spectra exhibit similar features at wave numbers characteristic of polysaccharides and the intensities of different bands vary noticeably or shift as a function of pH.

With changing pH of coagulation, there is an increase in the intensity of the band 3,400 cm^{-1} characteristic of hydroxyl groups (OH) elongation at pH$_{10}$. The second change was an appearance of a well-defined shoulder around 3,006 cm^{-1} and 3,009 cm^{-1} assigned to the symmetrical and asymmetric C–H aromatic group stretching vibration of the cis-double bond (=CH) (Vlachos *et al.* 2006)

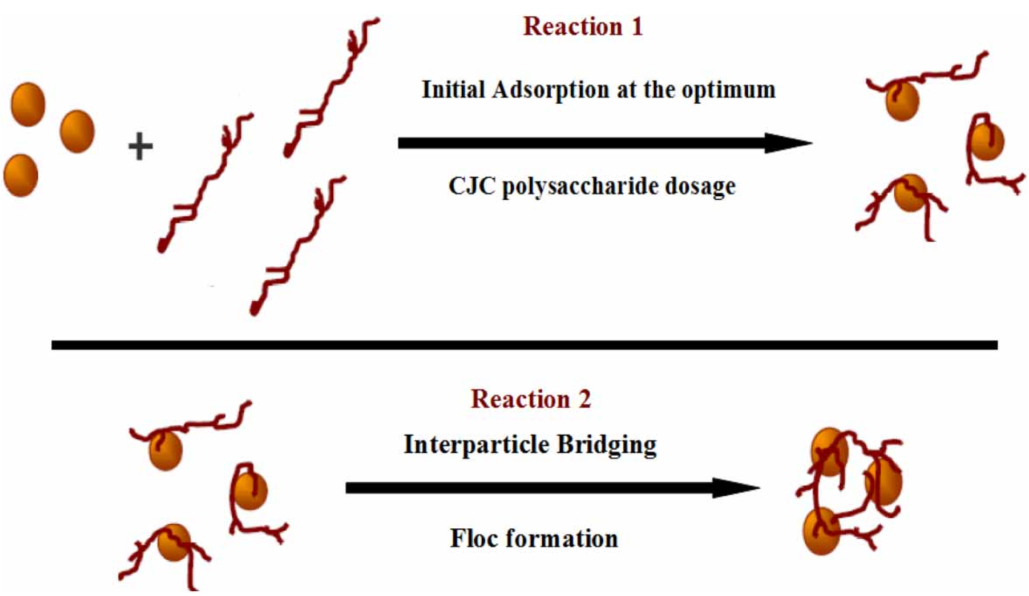


Figure 9 | Schematic representation of CJC polysaccharide and possible molecular interactions by adsorption and interparticle bridging.

Table 5 | Evaluation of the main FTIR absorption bands and assignment observed after cactus juice coagulation process

Typical wave number (cm^{-1})	Assignment suggested	Attributed stretching vibration of different bonds in different FTIR Spectrum			
		CACTUS	pH_2	pH_{10}	Reference
a. Region of hydrogens stretching					
3,500–3,100	O–H stretching, N–H stretching (minor), hydrogen-bonded OH, OH groups of alcohol and carboxylic acids	3,400	3,400	3,400	Santhiya *et al.* (2002), Zhao *et al.* (2007)
3,006–3,009	Stretching of aromatic C–H, C–H stretching vibration of the cis-double bond (=CH).	Absent	3,006	3,009	Ait Baddi *et al.* (2004), El Samrani *et al.* (2004)
2,920–2,955	C–H asymmetric stretching in CH_2, C–H asymmetric stretching in CH_3	2,934 2,955	2,926 2,955	2,926 2,955	Zhao *et al.* (2007), Gu *et al.* (1994)
2,850	C–H symmetric stretching in CH_2	2,850	2,854	2,854	Dignac *et al.* (2000), Guibaud *et al.* (2003)
b. Region of double bonds stretching					
1,725–1,743	C = O in COOH groups, C = O bond of non-ionic carboxylic acids and esters (–COOH, –$COOCH_3$)	1,720	1,738	1,743	Li *et al.* (2007)
1,650–1,544	Strong asymmetrical stretch of COOH, ionic carboxylic groups (–COO–), asymmetric/antisymmetric, aromatic C–C skeletal vibrations, C–O stretching of amide groups (amide I band), C–O of quinone and/or H-bonded, conjugated ketones	1,620	1,640	1,580	Farinella *et al.* (2007), Ricca & Severini (1993), Li *et al.* (2007)
1,540–47 and 1,507	N–H deformation and C–N stretching, (amide II band), aromatic C–C stretching,	1,515	1,514	Absent	Droussi *et al.* (2009)
c. Region of other bonds deformations and bendings					
1,462–1,380	Symmetrical stretching of C = O in the complexed carboxylate groups, O–H deformation and C–O stretching of phenolic groups COO– antisymmetric stretching, C–H bending of CH_2 and CH_3 groups	1,410 1,440	1,407 1,440 1,383	1,400 1,440	Manrique & Lajolo (2002), Ricca & Severini (1993) Blackburn & Burkinshaw (2002)
1,000–1,150	OH of alcoholic groups, C–O stretching of secondary alcohols	1,078	1,074	1,080	Crognale *et al.* (2006); Manrique & Lajolo (2002)

(Table 5). The appearance and/or increase in the intensity of these bands is due to dissolved organic matter removed by cactus juice. Absorbance peaks related to carboxylic functional groups also evolve distinctly with pH solution. The band at 1,720 cm^{-1} (C = O stretching vibration in COOH groups), presented by a distinct well-defined shoulder is replaced after coagulation in sludge by a better-resolved peak especially at pH_2. The same band is less intense at basic pH. However, the band at 1,410 cm^{-1} is shifted and increases sharply to reach a maximum to 1,400 cm^{-1} (symmetrical stretching of COO^-, OH deformation, and C–O stretching of phenolic groups) at pH_{10} (Table 5). It is widely known that pH affects the ionization of active sites on the biocoagulant surface (Rojas *et al.* 2005). In the cactus mucilage, carboxyl groups can exist as un-ionized molecules or as carboxylate ions. At an adjusted pH, the absorbance measured is the absorbance manifested by molecular and/or ionic forms. At pH_2 the COO^- is converted to COOH functional groups. Carboxyl groups are less involved in complexation reactions with cations hydrolyzed species since there is no charge complementarity (additional charge). The COOH accumulation in sludge leads to enhanced absorption of COOH groups with a well intense band in the FTIR spectra. Conversely, carboxylates ions complexed to cations hydrolyzed species are represented by weaker bands (Figure 8(b)). In the same context, Betatache *et al.* (2014) have calculated the zero point charge (pHzpc) of the mucilage of *Opuntia ficus Indica*; they found a pH of 4.3. At pH of the juice lower than this value, the surface of the dried cactus juice is positively charged, and when the pH value is higher than 4.3, the surface of the dried cactus juice is negatively charged (Betatache *et al.* 2014). This explains much more the low removal efficiency for suspended solids and organic matter obtained at acidic pH. At pH higher than seven, OH^- concentration increases and disrupts the equilibrium concentration of ions in the solution. Then the equilibrium shifts to the left and enables more protons from the carboxyl group to form water molecules. Accordingly, more COO^- adsorption sites in the polysaccharide backbone (essentially an anionic polyelectrolyte) are exposed, which acts as a chemical adsorption site for cations (Figure 8(c)) (Yin 2010).

Structure interaction between polysaccharide-polyphenols

In the case of polyphenol, they are two types of polyphenol–biocoagulant interactions: one electrostatic (like metallic ions) due to the ionizable hydroxyl group and other hydrophobic due to the aromatic ring that polyphenols present in their structure. Electrostatic interactions are proportionally maximized with increasing pH, while hydrophobic interactions will be favored as long as hydroxyl groups are still protonated. At pH_{10} we have the greatest removal percentage of polyphenols (Figure 7), although in the same solution there is the phenoxide anion and cactus mucilage rich in polysaccharide. Therefore, the carboxyl groups are not responsible for polyphenol adsorption in cactus mucilage, due to the repulsion of their negative charges. The adsorption properties of polysaccharides could be due to the existence of unique intermolecular interaction between the π-electron system of the aromatic compounds (polyphenols) and the OH^- group of the polysaccharide (Yoshida *et al.* 1964; Blackburn & Burkinshaw 2002; Yin 2010).

CONCLUSION

The results reported in this study show that the use of cactus juice as a coagulant for olive mill wastewater pretreatment is highly dependent on the pH and coagulant concentration in the solution. Under the optimal conditions of pH_{10} and coagulant concentration 10%, removal efficiency of COD (74%); color (λ_{465}; λ_{395}) (75%; 68%); TSS (89%); turbidity (93%) and polyphenols (51%) were achieved. FTIR spectra for different samples (lyophilized cactus juice rich in polysaccharides, and lyophilized OMW sludge after pretreatment at (pH_2) and (pH_{10})) revealed that mucilage of *Opuntia* spp. operates predominantly through adsorption and interparticle bridging coagulation mechanism. Finally, cactus *Opuntia* has a potential capacity to be used for OMW pretreatment applications. This approach affords a technical justification for using natural coagulants to treat olive mill effluent.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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