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Use of a New Bio-Flocculent Extracted from Moroccan Cactus in the Treatment of Polyphenol-Laden Waste by the Flocculation Coagulation Process

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ABSTRACT

Olive cultivation is currently the subject of great exposure (Ministry of Agriculture and Maritime Fishing, 2015), and as a consequence of the implementation of the national olive cultivation development programme, an area of 1,220,000 ha (+ 120%) and a total olive production of 2,500,000 tonnes/year (+ 70%) are among the objectives of the Green Morocco Plan by 2020. The olive sector has experienced a very important movement in the context of the 2017–2018 campaign, which Morocco should record a volume never reached before, with a production record estimated at 1.56 million tons, the production of this campaign shows an increase of 47.8% compared to the previous season. In this work, we determined the efficiency of a new biodegradable natural flocculant extracted from the prickly pear Opuntia ficus indica in a physico-chemical process by coagulation-flocculation, to treat liquid discharges loaded with organic matter and phenols difficult to degrade. The tests were carried out on six well-preserved samples subjected to increasing concentrations of coagulant and flocculant after adjustment of the pH. The results obtained are very encouraging for this type of physico-chemical treatment and work is still in progress until there is a significant improvement in the rate of abatement of the pollutant load.

Keywords: natural flocculant, Opuntia ficus indica, coagulation-flocculation.

INTRODUCTION

The olive industry, whose final product is olive oil, in a ranking where countries are rated from 1 to 5, the latter expressing the highest level of water stress, Morocco was awarded a score of 4.68, placing him in 19th position [1].

This industry generates two residues, one solid: the pomace (residues of the pulp, fragments of cores), and are reused in agriculture and industries (food industry, production of soaps) [2] and the other liquid, vegetable water (water contained in the olive, washing water, water related to the treatment process) [3], the latter are often discharged into the receiving environment. without any treatment, which harms the environment by causing pollution of water, air and soil, because of the high organic load that requires high oxygen consumption.

The toxicity of these vegetable waters [4] is mainly due to the presence of long-chain free fatty acids and phenolic compounds at high concentrations (4 to 15 g/l), phenolic compounds, organic acids and high salinity (high conductivity) can cause phytotoxic effects on olive trees; they cause a decrease in the dry matter by decreasing the availability of nitrogen, in addition to their toxicity for certain microorganisms. Acids, mineral elements and organic substances result in a destruction of the cation exchange capacity of the soil (CECS), hence a reduction in soil fertility. This causes the non-biodegradability of vegetable water, these considerations have has led several researchers nationally and internationally to choose treatment routes or valorisation of vegetable water to limit this pollution [5], However, the processes developed so far are very limited and their cost very high. However, the possible treatment methods for the removal of the charge treatment by coagulation-flocculation processes seems to be one of the best techniques to use because of its simplicity and efficiency.

Several metallic chemical coagulants are used [6]. But these products have a probative toxicity to the environment, which prompted researchers to test coagulants and flocculants of natural origin to achieve the coagulation-flocculation process.

We have chosen for our experimental work to develop a cactaceous plant that grows in different regions of Morocco, to extract a natural bio-flocculant, available, biodegradable in the treatment of vegetable waters by coagulation-flocculation process, the latter could be a good alternative for the depollution of polluted waters.

MATERIAL AND METHODS

The experimental study was carried out in the Laboratory of Materials and Environment Engineering (LMEE) of the Sidi Mohamed Ben Abdellah University, Faculty of Sciences Dhar El Mahraz, Fez.

Sampling

The vegetable waters studied are taken from a modern olive-milling unit in the Fez region, then stored at 4 °C in the dark until they are used.

Characterization

The physicochemical characterization of vegetable water was performed by measuring a number of parameters namely pH, electrical conductivity (EC), dry matter (DM), biological oxygen demand, chemical oxygen demand (COD) and phenolic compounds according to AFNOR standards issued by Rodier. This characterization concerns raw vegetable water and diluted 1/10.

- pH – the pH was measured using the pH-meter type HANNA instruments after calibration buffer solutions of pH = 4, pH = 6.86 and pH = 9.18. The products used to adjust the pH are: Sodium Hydroxide (NaOH) and Hydrochloric Acid (HCL).
- dry matter – the dry matter consists of all the organic and inorganic substances, in solution or suspension, contained in the vegetable waters. The principle consists in placing a mass of the sample (MB) in a crucible and introducing it into an oven at 105 °C. After the sample is weighed again until a constant mass is obtained (MS). The dry matter content is calculated by the following relation (1):

$$MS = \frac{m - m_0}{m} \times 1000 \tag{1}$$

where: MS – dry matter content (g/L);
m_0 – mass of the empty beaker;
m – mass of the beaker and margines after drying.

- temperature – we measured the temperature using a HANNA instruments pH meter, having two electrodes, one for temperature and the other for measuring pH.
- electrical conductivity – the electrical conductivity was measured by a conductivity meter HANNA instrument, it is expressed in mS.cm-1 which depends on the temperature of the medium.
- chemical oxygen demand (COD) – the COD corresponds to the consumption of oxygen necessary for the complete oxidation of the organic matter of the vegetable waters, expressed in gram of oxygen per liter of sample. The determination of the COD is carried out by the potassium dichromate method. The method is based on the principle of oxidizing the organic matter (150 °C for 2 hours), with a standard solution of potassium dichromate $K_2Cr_2O_7$ in the presence of sulfuric acid. Potassium dichromate is preferred over other oxidants because of its powerful oxidizing power, versatility, purity and high stability [7].
- biological oxygen demand (BOD_5) – BOD5 is the amount of oxygen required by aerobic microorganisms in water to oxidize organic matter, dissolved or suspended in water, determined by the respirometric method in a thermostatically controlled chamber (AFNOR, T 90-103), in darkness and for 5 days using a BOD-meter. The incubation of the sample lasts five days in the dark and at a temperature of 20 °C. The values of BOD_5 are expressed in mg O2/L [7].

- total phenols – the phenolic compounds of the various extracts obtained are quantified by measuring the absorbance at the maximum wavelength 725 nm whose intensity is proportional to the amount of polyphenols present in the sample using the Folin-ciocalteu method, described by [8]. The content of phenolic compounds is determined from a calibration curve obtained using gallic acid solutions at different concentrations.
- calibration curve
  - preparation of the calibration range
    - prepare a solution of 4.8 g of gallic acid;
    - dissolve in 1000 ml of distilled water;
    - dilute the solution to have different concentrations (see Table 1).
  - determination of total polyphenols;
    - pollowing samples of raw and diluted vegetable water;
    - praise 1 ml of each sample;
    - dilute 50 times with distilled water;
    - prave 1 ml of each dilution;
    - add 5 ml of distilled water to each flask;
    - add 1 ml Folin reagent ciocalteu;
    - add 1 ml of 20% sodium carbonate after 3 min;
    - incuber at room temperature and away from light for one hour;
    - agitate and let sit for an hour.

The reading of the absorbances is made at the maximum wavelength 725 nm and the concentration of total phenolic compounds is determined by reference to the calibration curve obtained and the polyphenol content is calculated by the following relation (2):

$$X = \frac{Y}{0.7648} \tag{2}$$

where: X – absorbance;
Y – the concentration of phenolic compounds.

The microbiological analyzes of the vegetable waters focused on the enumeration of yeasts and molds (L and M), and bacteria.

Table 1. Dilutions of gallic acid at different concentrations

Gallic acid concentration g/L	4.5	3.6	2.7	1.8	0.9
Dilution	5/5	4/5	3/5	2/5	1/5

Preparation of the bioflocculant

The prickly pear is a plant native to Mexico introduced in North Africa in the 17th century by the Spanish and mainly grown for the production of its fruits [9]. Adapted to arid and semi-arid zones [10], it is an interesting plant because of the environmental conditions in which it develops and its resistance to extreme climatic conditions [11], it belongs to the cactus family and more specifically to the genus Opuntia. The Opuntia genus contains about 300 species and many of them produce very tender and edible stems and fruits [12]. Among these species, Opuntia Prickly pear.

The barbaric fig tree, which grows abundantly in Morocco in several arid and semi-arid regions, has been collected in the vicinity of Taounat region (Figure 1).

The bio-flocculant used in our trials is extracted from the barbaric fig racket (*Opuntia ficus indica*). It is tested for its flocculation ability and it can retain its flocculant capacity outside of any preservation system.

Snowshoe powder from Opuntia ficus indica

For the production of the powder we have followed the following steps:

Figure 1. *Opuntia ficus indica*

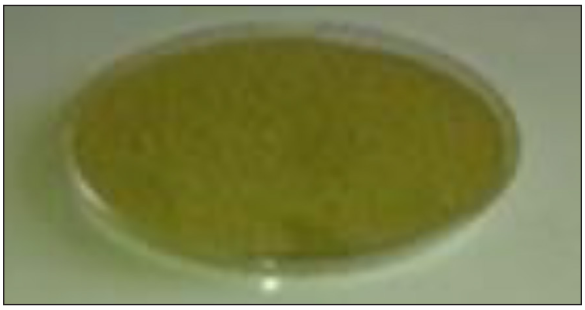

Figure 2. Snowshoe powder of *Opuntia ficus indica*

Figure 3. Snowshoe juice of *Opuntia ficus indica*

- snowshoe cleaning;
- 400 g drying in an oven at a temperature of 90 °C for 24 hours;
- grinding dried snowshoes (24.38 g) in a grinder;
- sieving to obtain particles of very fine powder (Figure 2).

The product obtained is a green coloring powder.

Snowshoe juice from *Opuntia ficus indica*

The extraction of the organic flocculant was carried out according to the following steps:

- snowshoe cleaning;
- mixing 200 g of the racket with 100 ml of distilled water;
- filtration (see Figure 3).

This product obtained (250 ml) is a viscous liquid miscible with water, green color and pH = 6.5.

Phytochemical characterization

With regard to quantitative chemical analyzes, tests have been carried out for the search for some chemical compounds of a mineral nature.

Treatment of vegetable waters by coagulation-flocculation

The polyphenols and organic matter that exist in our effluent were removed by coagulation flocculation using a Jar-test system (Figure 4), Several tests were carried out on 1/10 diluted vegetable water, to evaluate the effectiveness of the developed biomaterial and to determine the optimal conditions of the pH, the concentration of the bio-flocculant and the stirring speed and the time of the contact.

RESULTS AND DISCUSSIONS

Our goal is to treat vegetable waters diluted 10 times by a biofloculant either in powder form or in the form of juice under well-defined operating conditions.

After a characterization of the studied samples, we carried out a follow-up of the various physicochemical parameters (COD, BOD_5, MS and polyphenols) during treatment.

Figure 4. Jar-test device

Table 2. Physico-chemical characteristics of the vegetable waters studied

Parameter	Margins brutes	Margins diluted 1/10
pH	3.93	4.23
Conductivity	9.9	7.8
Temperature	25	25
Dry matter(g/L)	50.38	20.24
DCO (g O_2/L)	68.86	19.30
DBO5 (g O_2/L)	45.50	27
Polyphenols (g/L)	3.32	1.56

Physico-chemical characteristics

Before carrying out the treatment tests, the effluent was characterized (Table 2):

According to the results obtained, it can be seen that the effluents studied are characterized by a very high acidity, which is due to the richness of the phenolic acids, fatty acids, by a high electrical conductivity which is due to the salting practiced to preserve the olives until trituration, by the organic matter load expressed in terms of chemical oxygen demand COD and biological oxygen demand BOD_5, These effluents are also characterized by the predominance of phenolic compounds which are responsible for the reddish brown color. After dilution, the pH increases but the other parameters decrease.

Enumeration of microorganisms

The results are illustrated in the Table 3. The results obtained show the presence of microoganisms such as mushrooms and yeast (Figure 5), are able to resist more than bacteria in vegetable water because they have antibacterial activity due to the presence of phenolic compounds, fatty acids, tannins and brown pigments.

Table 3. The concentrations of microorganisms presented in the vegetable waters studied

Microbial flora	UFC/ml
Mushrooms	$5.40 \cdot 10^5$
Yeasts	$7 \cdot 10^5$
Bacteria	0

The total absence of bacteria is due to the acidity of vegetable water and the activity of polyphenols.

Characterization of the bioflocculant

Studies have shown that the mineral composition (Table 4) is 50 mg/100g dry weight, 18–57 mg/100g dry weight for potassium, 11–17 mg/100g for calcium and magnesium, followed by manganese (62–103 μg/g), iron (59–66 μg/g), zinc (22 to 27 μg/g) and copper (8–9 μg/g) are in agreement with the results found by [13].

The bioflocculant *Opuntia ficus indica* is very rich in calcium and potassium. The main amino acids are glutamine, followed by lysine, valine, arginine, phenylalanine and isoleucine [14].

Table 4. Content of some compounds of the mineral fraction

Mineral	% dry matter
Ca	4.7
K	4.6
Na	0.0
Mg	1.0
Fe	0.05
Mn	0.5
Zn	0.5
Pb	0.01
Cu	0.02

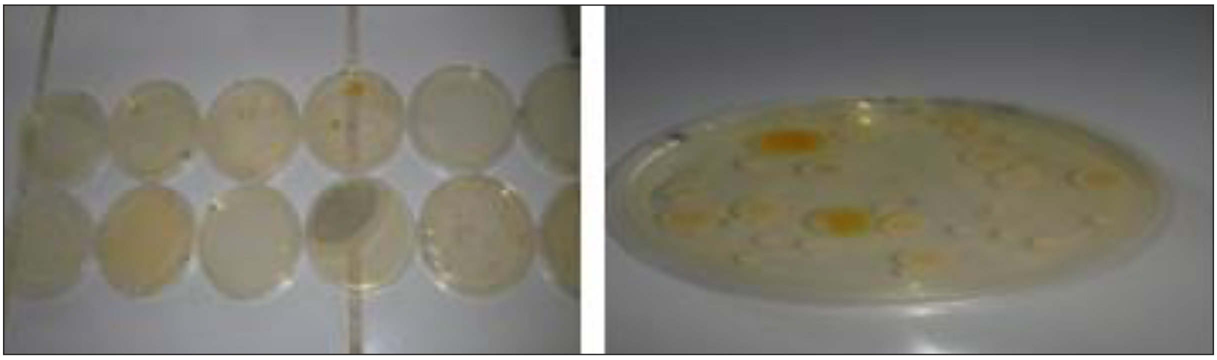

Figure 5. Mushrooms and yeasts obtained

Treatment of vegetable waters

pH adjustment

We seek to find the optimum pH value of the powder for the treatment of vegetable waters by the coagulation / flocculation process under well-defined operating conditions:

The concentration of the coagulant = 15 g/L; Coagulation stirring speed = 200 rpm for 3 min; Flocculation stirring speed = 20 rpm for 20 min (see Figure 6).

The optimum pH value in the case of powder is 6.5. At this value the negative charge of colloids is neutralized which allows an increase in the rate of abatement.

Optimization of the biofloculant concentration: Opuntia ficus indica powder

Efforts have been made to treat the margines with Opuntia ficus indica powder alone, as a bio-flocculent, and to study their effect on organic matter with a pH value of 6.5, and the polyphenol reduction ratio, BOD_5. COD, and MS (Figure 7, Figure 8). It is clearly seen in Figure 7 when the concentration of opuntia ficus indica powder is 15 g/L, there is a parametric reduction of COD, BOD_5 as well as MS respectively the following values 10 g/L, 18 g/L, 10 g/L while in a concentration of 22 g/L, the polyphenols reduced in 1.35 g/L, this result is confirmed by the results obtained by

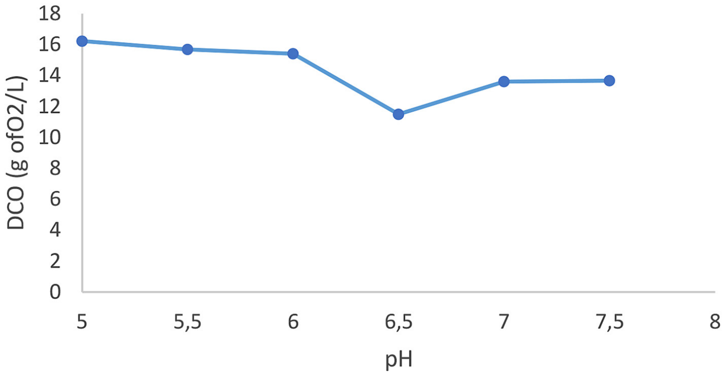

Figure 6. Evolution of COD as a function of pH

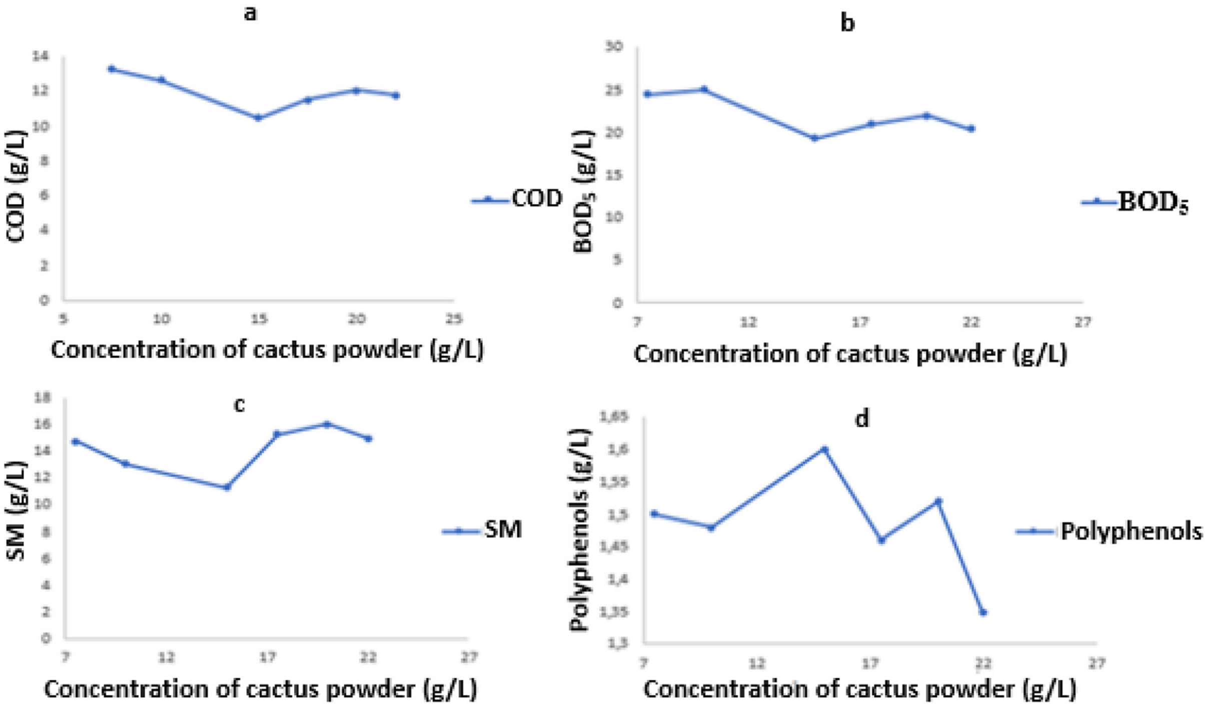

Figure 7. (a) Variation of COD as a function of the concentration of the powder, (b) variation of BOD_5 as a function of the concentration of the powder, (c) variation of MS as a function of the concentration of the powder, (d) variation of polyphenols according to the concentration of the powder

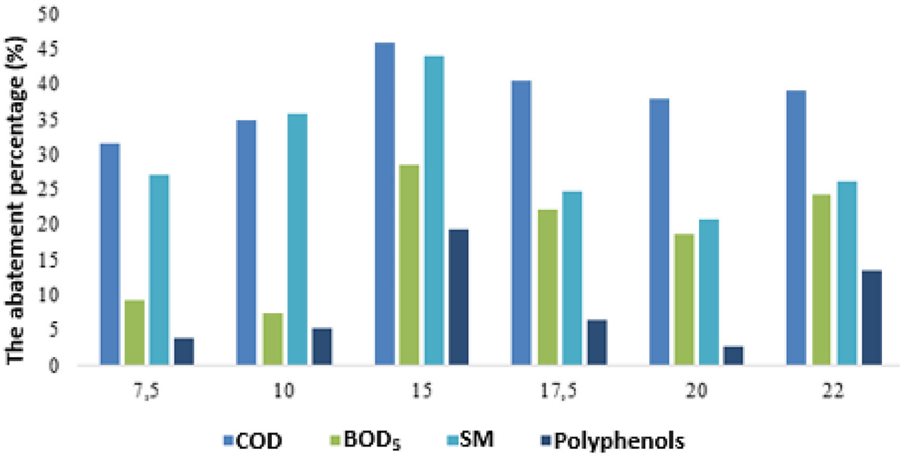

Figure 8. Change in the abatement rate (%) of MS, COD, BOD_5 and total polyphenols as a function of powder concentration

N.sadif [21], while the greatest abatement percent is for chemical oxygen demand (Figure 8).

The use of our plant in powder form at a concentration of 15 g/L gives good performance in terms of the various parameters measured or will have a COD reduction rate of 45.90% and a removal efficiency of BOD_5 of 28.51%, the MS of 44.07%, the abatement rate of polyphenols is slightly low of about 19.23%

Case of the biocoagulant – the juice of Opuntia ficus indica

The results of using Opuntia ficus indica juice in the treatment of vegetable waters is illustrated in Figure 9 at a stirring speed of 200 rpm for 3 min for coagulation and 20 rpm for 20 min for the treatment. flocculation with pH = 6.5 (Figure 9).

We clearly notice in Figure 9 a parametric reduction COD, BOD_5, suspended matter as well as polyphenols in a volume of cactus juice of 20 mL, this reduction is much less important than that obtained by the powder, several works show the effectiveness of this plant and especially the powder better than the juice. This result confirmed by Zohri [22] who showed that the cactus extract has a good efficiency in the treatment of industrial water, even without the addition of flocculant.

From the results obtained in terms of the abatement rate as shown in the Figure 10, we find that the addition of juice displays a reduction

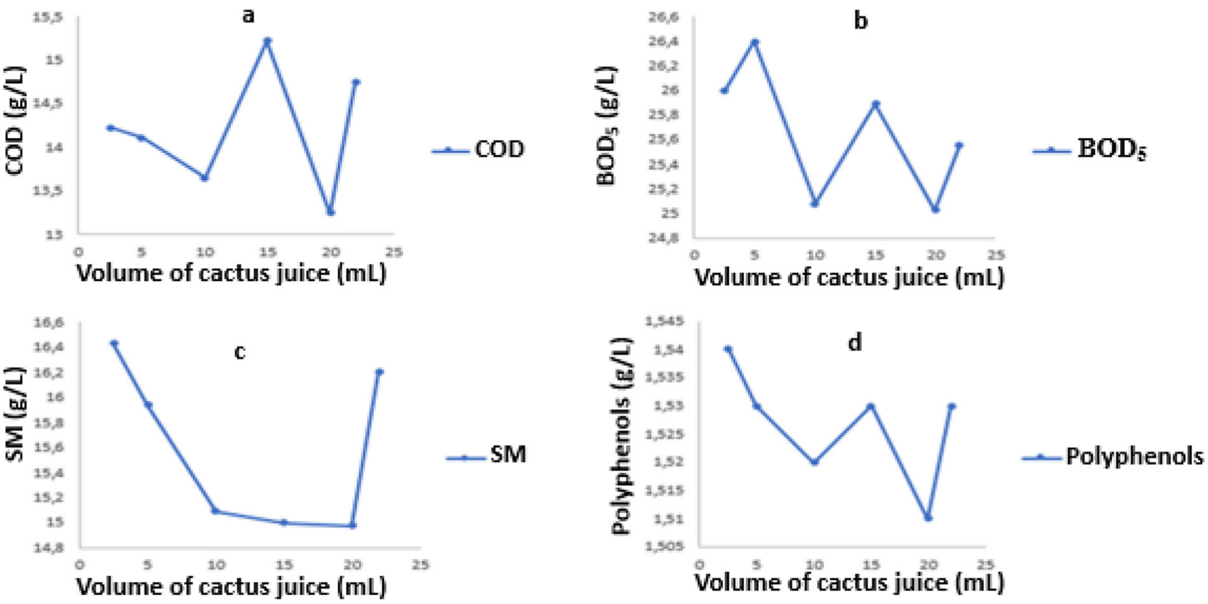

Figure 9. (a) Variation of COD as a function of volume of juice, (b) variation of BOD_5 according to volume of juice, (c) variation of MS as a function of juice volume, (d) variation of polyphenols according to volume of juice

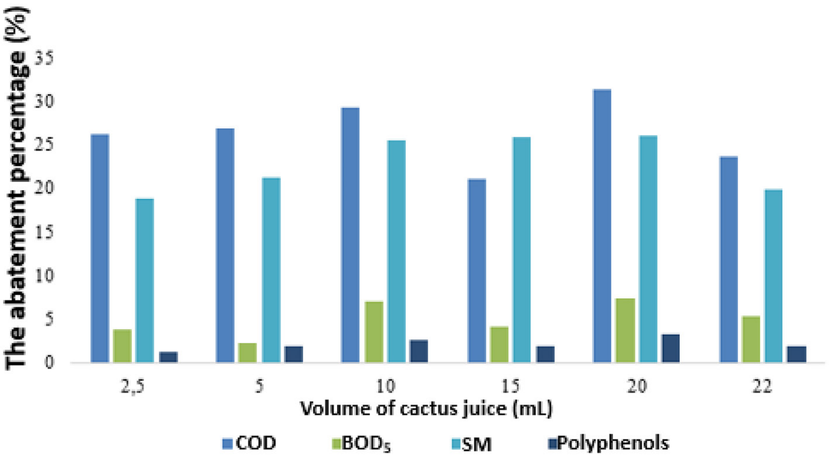

Figure 10. Change in the rate of abatement (%) of DM, COD, BOD_5 and total polyphenols as a function of cactus juice concentration

percent of COD, BOD_5, SM, polyphenols of the order of 31.34%, 7.29%, 25.98%, 3, 20% respectively. We have noticed that the juice has no capacity to adsorb a large pollutant load compared to *Opuntia ficus indica* powder.

Combination between the coagulant (lime) and the bioflocculant (the powder of Opuntia ficus indica)

After the treatment of vegetable waters with aluminum sulphate with the powder, treatment tests were carried out by the combination of lime and powder to find out which is the most effective treatment with fixation of all the parameters of the process and variation in the concentration of Opuntia ficus indica powder from 7.5 g/L to 22 g/L (Figures 11 and 12).

The results show that there are two phases, an increase phase of 7.5 to 15 g/L and a reduction phase of the abatement rate which could be explained by the reversal of the charge. colloidal particles.

At a concentration of 15 g/L a very good result has been obtained which is expressed by

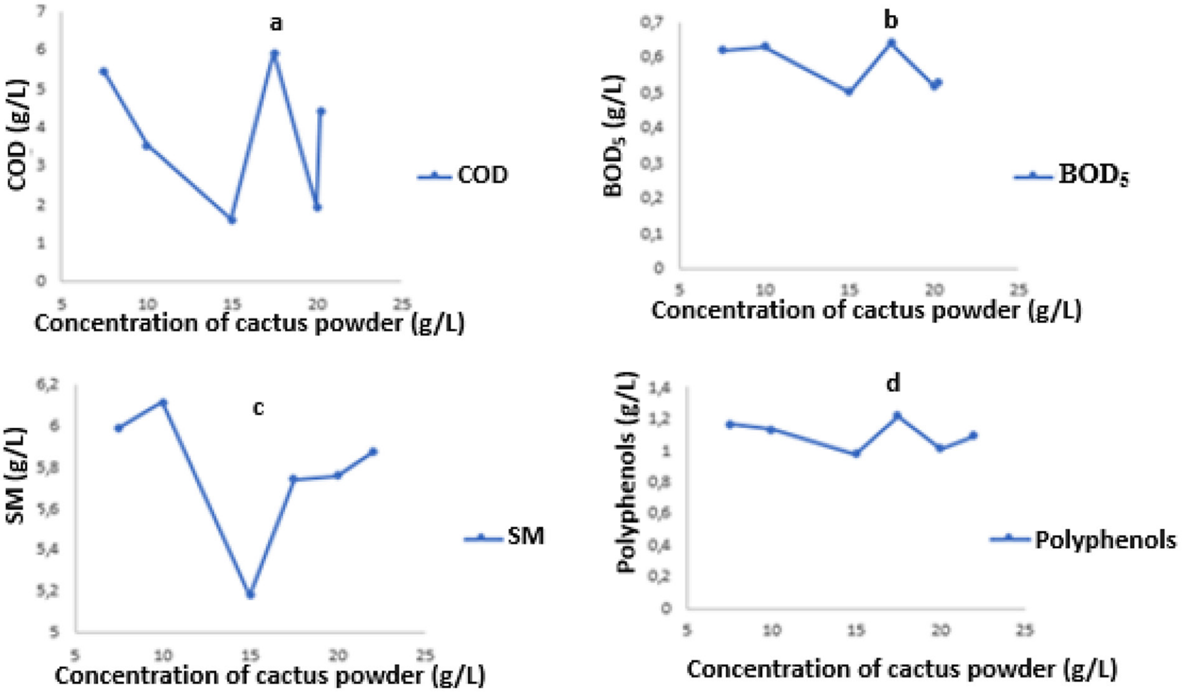

Figure 11. (a) Variation of the COD as a function of the cactus powder with fixation of the lime, (b) variation of the BOD_5 as a function of the cactus powder with fixation of the lime, (c) variation of MS as a function of cactus powder with fixation of lime, (d) variation of polyphenols as a function of the concentration of the cactus powder with fixation of the lime

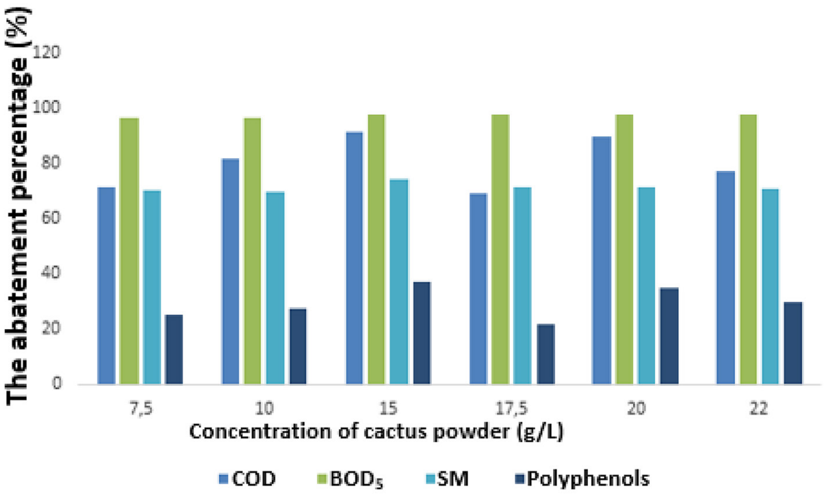

Figure 12. Change in the abatement rate (%) of SM, COD, BOD_5 and total polyphenols as a function of cactus powder concentration with fixation of lime concentration

increasing the rate of reduction of COD, BOD_5, MS and polyphenols which is of value of 91.70%, 98.14%, 74.38% and 37.17% respectively.

The snowshoe powder of *Opuntia ficus indica* has a very good ability to flocculate with milk lime while avoiding toxicity to the environment and human health. Cactus powder is a biomaterial with interesting adsorption capabilities that can be an alternative to other commercial media. The experimental results showed that the adsorption of the Methylene Blue dye on the Cactus reaches 61% at initial pH and at room temperature [15].

Tests carried out on tannery effluents make it possible to conclude the effectiveness of an extract of racquet of Opuntia ficus indica (juice or powder) by the coagulation-flocculation process for the depollution of a liquid rejection notably for the reduction of the turbidity, metal ions or organic fillers, BOD5, COD and suspended solids. Thus, the treatment of the textile effluent shows a good flocculation performance by powder or juice. Elimination of copper in the supernatants reached 99.6% and more while turbidity decreased by 98%. BOD_5, COD and suspended solids follow the same fall pattern [16].

The study was carried out on samples loaded with Zinc and Suspended Materials (MES). The treatment is based on coagulation with lime, followed by flocculation with our new product. The latter showed a very significant effect on the elimination of Zinc and MES with a yield of around 96% and 99%, respectively [17]. Similarly, [18] used bioc flocculant extracted from Moroccan cactus in the treatment of chromium (VI) loaded rejects by the flocculation coagulation process [19].

Optimization of the stirring speed

After optimizing the pH and concentration of the various coagulants and flocculants, several

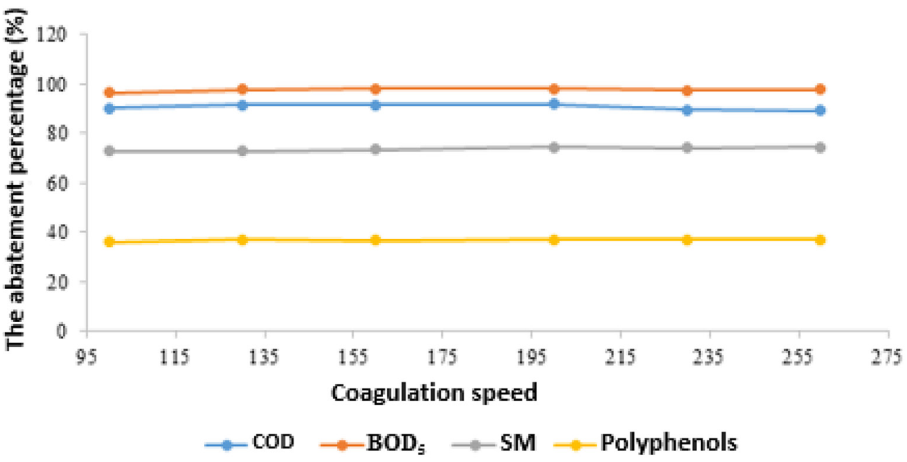

Figure 13. Variation of the organic load as a function of the coagulation rate

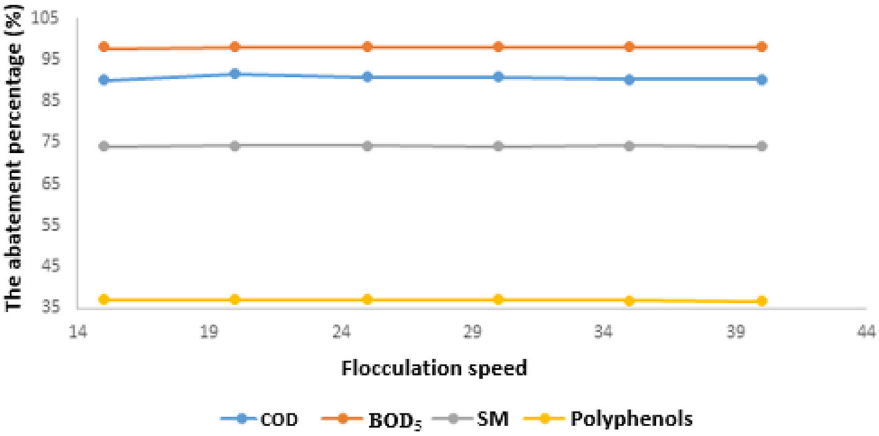

Figure 14. Variation of the organic load as a function of the flocculation rate

attempts were made to optimize the agitation rate of the coagulation flocculation of vegetable waters diluted 1/10 (Figures 13 and 14).

From the results of the optimization of the coagulation stirring speed, we have noticed that 200 rpm is the ideal speed for the neutralization of the colloid charges. On the other hand, the ideal flocculation stirring speed is 20 rpm.

Optimization of stirring contact time

Among the factors that affect the flocculation coagulation process, there is the contact time that is short for coagulation and slow for floc formation (Figures 15 and 16).

After several attempts, it was obtained as results that the optimal contact time for the

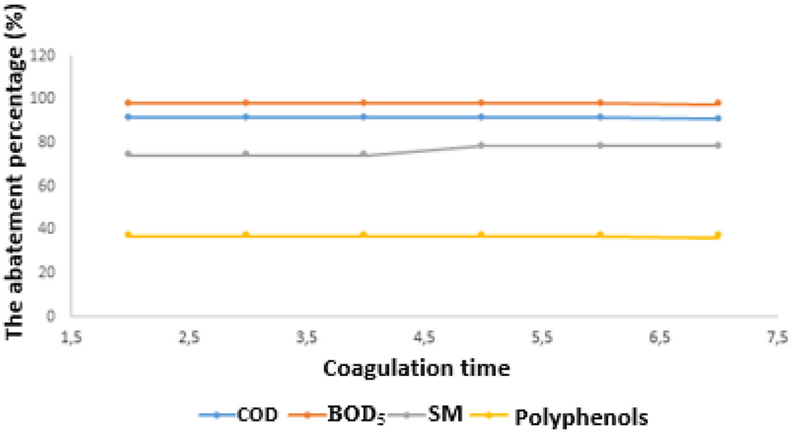

Figure 15. Variation of the pollutant load as a function of coagulation time

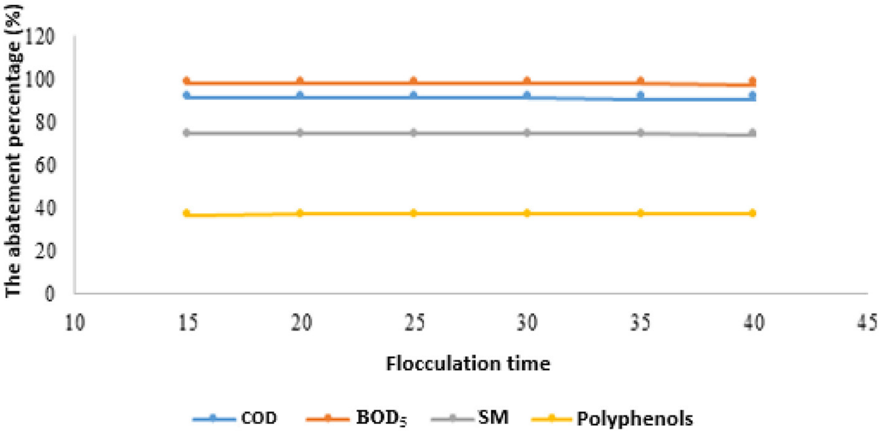

Figure 16. Variation of the pollutant load as a function of flocculation time

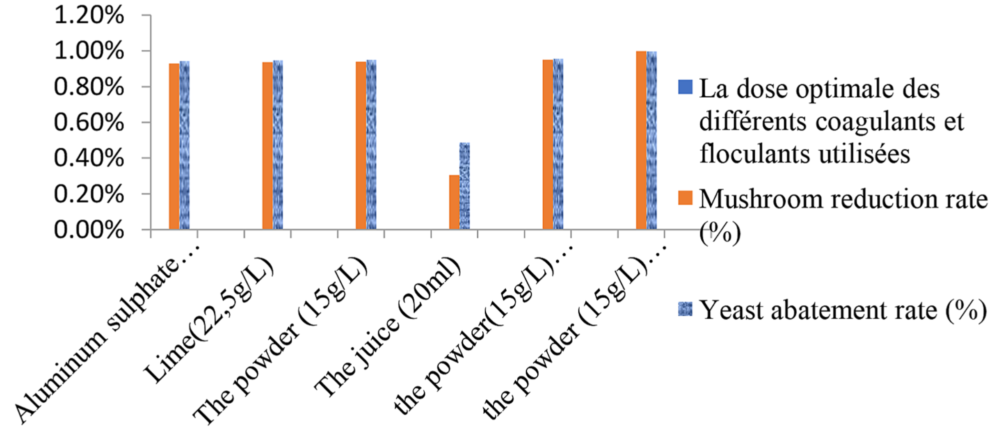

Figure 17. Enumeration of microorganisms after treatment of vegetable waters

neutralization of the charges is 3 min; on the other hand, the flocculation requires 20 min.

Monitoring of microorganisms during treatment with different materials

Studies show the total absence of pathogenic microorganisms in vegetable water, so these effluents do not pose problems on human health. Microbiological analyzes have shown that vegetable waters have antimicrobial activity against yeasts and fungi, support the high salinity and acidic pH characteristics of these effluents, and resist more than bacteria to phenolic substances [20, 21].

The present study is devoted to the enumeration of the microbial load of vegetable water to establish the link between the presence and absence of microorganisms and the effect of phenolic compounds [22, 23].

We followed the enumeration of the microorganisms under operating conditions whose optimal dose of the powder is 15 g/L, the optimal dose of juice is 20ml and the lime 22.5 g/L (Figure 17).

After the microbiological tests there is a significant reduction of fungi and yeasts due to the change in growth conditions the increase in pH and decrease in organic load [24, 25]. This gives a better elimination of the colloidal particles and toxic substances responsible for the inhibitory effect of vegetable water with a reduction of the microbial load [26].

The results have been very promising in this treatment and the work is still in progress with the aim of improving the yield.

CONCLUSION

The coagulation-flocculation process is often complex and strongly dependent on pH, coagulant and flocculant dose, agitation rate and contact time. Most of the work leads to the conclusion that the optimization of physico-chemical parameters can lead to an increase in the rate of abatement of organic matter accompanied by an increase in the volume of sludge.

The valorization of a cactaceous plant as a biofloculant in the treatment of vegetable waters could be a natural ecological alternative, effective and less expensive, unlike its chemical counterparts which have negative effects on the environment, despite their effectiveness in the treatment of liquid discharges.

We were able to conclude that:

- pH plays a determining role in coagulation,
- the determination of the optimal concentration of the coagulant on Jar-test is an essential parameter for the destabilization of the colloids and the formation of the flocs,
- coagulation requires very fast speed and short time,
- flocculation requires very slow speed and time,
- *Opuntia ficus indica* powder gives better results compared to other industrial products.

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