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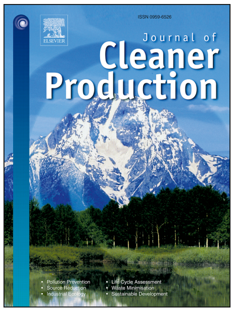


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**Reuse of olive mill wastewater as a bioflocculant for water treatment processes**

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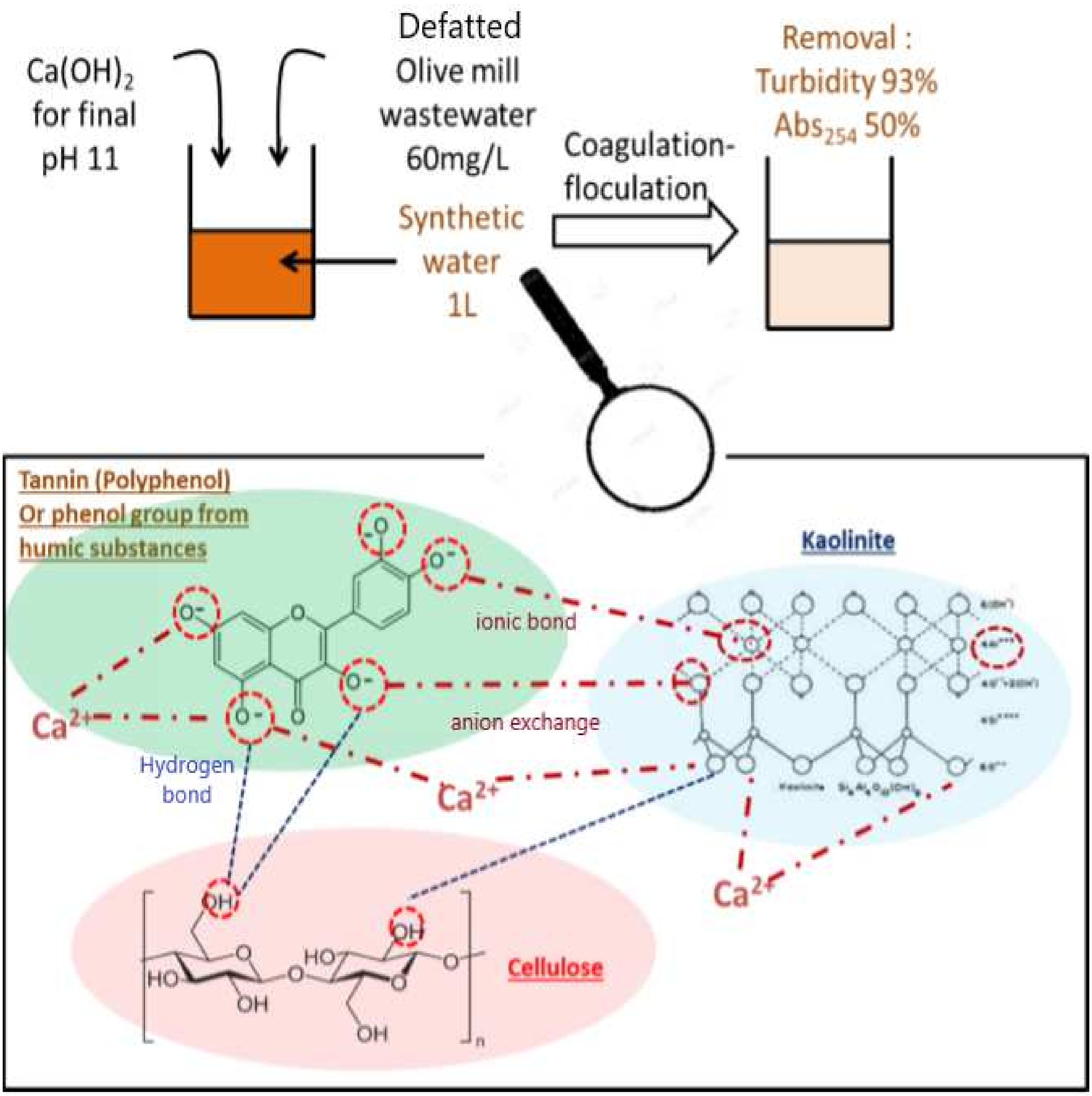
**Graphical Abstract**



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## Abstract

This study investigates the reuse potential of olive mill wastewater (OMW) high in tannin content and whose complete treatment requires the use of complex and expensive processes. Tannins from OMW could serve as bioflocculant compounds according to the literature. The defatted OMW is tested in a coagulation-flocculation process on synthetic water, and the best turbidity removal rate derived is: 92% ± 1% at pH 11 for 100 mg $L^{-1}$. Water-solubilized material at pH 11 precipitates after acidification at pH 6. This precipitate fraction (PP), solubilized at pH 11, is found to contain more active constituents with a turbidity removal rate of 82% ± 2% for a 60 mg $L^{-1}$ dose yet reveals an increase in absorbance at 254 nm. This increase in absorbance can be corrected by using lime for a pH adjustment to 11 with an absorbance removal at 254 nm of 50% ± 1%. The bioflocculant material is characterized by means of physicochemical methods. Acid-base titration, enzymatic treatment and colorimetric dosage all confirm that tannins and/or flavonoids and cellulose constitute the active groups involved in the coagulation-flocculation process.


**Keywords:** bioflocculation; olive mill wastewater; tannins; flavonoids; cellulose; reuse.

## 1. Introduction

Olive mill wastewater (OMW) is a byproduct of the three-phase process of olive oil extraction, with a global annual production reaching 8.4 million $m^3$ (Doula *et al.*, 2017). This black liquid is composed of the olive fruit vegetation water, the water used for washing and treatment, plus a portion of the olive pulp and residual oil (Scioli *et al.*, 1997). Contamination from this waste can be reduced by adsorbing polyphenols onto activated carbon (Annab *et al.*, 2018). However, a complete treatment of OMW had only been possible using expensive advanced oxidation processes (Ochando-Pulido *et al.*, 2017) or electrocoagulation (Flores *et al.*, 2018). Recovery methods have been proposed, such as the production of $H_2$ yield by means of supercritical water gasification (Casademont *et al.*, 2018) or by reaching much greater depths in agricultural practices (Pardo *et al.*, 2017) depending on the potential processed wastes (Campani *et al.*, 2017).

OMW is a foul-smelling acidic liquid composed of water (83-92%), organic matter (4-16%) and minerals (1-2%) (Perez *et al.*, 1998). The organic load, as reflected in both the high biological oxygen demand (BOD) (up to 100 g $L^{-1}$) and chemical oxygen demand (COD) (up to 200 g $L^{-1}$), contains mostly sugars, polyphenols and fats (Hamdi *et al.*, 1993). The use of OMW as organic compost without any treatment could cause a degradation of the soil or water, due in particular to its phenolic constituents (antimicrobial activity) as well as to the low pH range (4-5) (Elhajjouji *et al.*, 2007). OMW contains a wide variety of compounds, such as polyphenol, polysaccharides, tannins and proteins (Michael *et al.*, 2014) and therefore is potentially involved in the coagulation-flocculation mechanism.

The coagulants and flocculants currently required for water treatment, i.e. metal salts and/or synthetic polymers, may exert several environmental impacts, namely: i) a possible increase of the metal concentration in water (with implications for human health); and ii) extensive sludge production. This production of metal hydroxides lies at the origin of many environmental investigations, due to the volumes involved and the high treatment-related costs if landfill burial is the chosen option. For these reasons, alternative coagulants and flocculants have been considered for environmental applications (Kudryavtsev and Kudryavtsev, 2016). Biopolymers may be quite attractive since they are vegetal products, and their life cycle could incorporate agricultural reuse or improved integration into the environment with biological degradation.

Some of the reported biological materials, including biopolymers (starches, chitosan, alginates), have shown their potential in the flocculation process. The biochemical families extracted from plants can also be used; these families include polysaccharides from *Ocimum basilicum* (Shamsjenati *et al.*, 2015), starches from cereal (Choy *et al.*, 2016), proteins from *Cocos nucifera* (Fatombi *et al.*, 2013), *Moringa oleifera* (Dolto *et al.*, 2018), polyphenols from *Opuntia ficus indica*, acorn (Bouaouine *et al.*, 2018a), *Cassia obtusifolia* (Subranmoniam *et al.*, 2014) and grape seed (Jeon *et al.*, 2009). Some polyphenols form complexes with macromolecules and, more specifically, with proteins via hydrogen bonds or hydrophobic interactions (Bruneton *et al.*, 2009). Polyphenols have already been introduced into water treatment as flocculants. Jeon *et al.* (2009) demonstrated that catechin (flavonoid) and tannic acid (tannin) were involved in the molecular flocculation mechanism. A commercial tannin-based flocculant product enhanced the performance of clarification in a biological treatment unit (Hameed *et al.*, 2018).

According to the literature, no information exists to date regarding the efficiency of OMW as a bioflocculant for water treatment. Given this context, the objectives of the present study are threefold: i) highlight the capabilities of OMW as a coagulant/flocculant; ii) enhance current knowledge on the most active constituents potentially involved in water treatment; and iii) propose an application in the field of water treatment. To achieve these objectives, water-soluble chemical compounds have been isolated. These materials were first characterized using overall physicochemical methods (zeta potential, colloidal particle size distribution and spectroscopy). The active molecular groups were then determined by means of acid-base titration, selective enzymatic treatment and colorimetric dosage.

## 2. Materials and methods

### 2.1 Bioflocculant preparation

#### 2.1.1 Preparation of olive mill wastewater materials

The olive mill wastewater (OMW) used herein was extracted from a modern semi-oil mill in Fez (Morocco); moreover, no chemical additives were used during production of this olive oil. In an initial step, the lipid residual was extracted from the concentrated suspension by organic solvents. These solvents (60 mL (2/1, v/v chloroform/hexane)) were then introduced into a suspension of OMW (100 mL) and shaken for 2 hours at room temperature. This step was repeated six times in order to ensure maximum recovery of the lipid. The defatted material (defatted OMW) was dehydrated at 80°C for 24 hours and stored at 4°C following freeze-drying (see Fig. 1). The volatile dry weight (% VDW) equaled: 78% ± 1%.

#### 2.1.2 Extraction at various pH levels

To separate the active constituents, 1 g of defatted OMW material was solubilized in 100 mL of distilled water at pH 6 and 11 adjusted with NaOH (1 M) and HCl (1 M). The Water Solubilized Material (WSM 6 and WSM 11) was then stirred at 200 rpm at room temperature for 24 hours ± 0.2 hours and filtrated at 0.45 µm, before being freeze-dried and stored at 4°C (Bouaouine *et al.*, 2018a). Next, WSM 11 was acidified to pH 6, which allowed precipitating the soluble alkaline extract under acidic conditions. The precipitate filtrated at 0.45 µm and re-solubilized under an alkaline condition (pH 11) was denoted "precipitate" (PP). This PP was also freeze-dried and stored at 4°C (Fig. 1).

## 2.2 Physicochemical characterization of defatted OMW materials

WSM 6 and 11 and the defatted OMW solution were subjected to 105°C for 24 hours in order to determine the dry weight (DW) of the material, in g $L^{-1}$. The volatile dry weight (VDW) was deduced after burning the previous sample for two hours at 550°C. VDW% is expressed as a fraction of DW. The residual solid encompasses all insoluble solids present in solution after a 0.45-µm filtration.

The UV-1800 Shimadzu spectrophotometer was used for the absorbance measurement campaigns. Absorbances at 210 and 280 nm were read in a glass cuvette after filtration (0.45-µm cellulose acetate) of 10 g dry weight of a defatted OMW fraction in 1 L. The majority of organic molecules absorbed at both 210 nm (carbohydrates, proteins, tannins, etc.) (Her *et al.*, 2008) and 280 nm (absorption of aromatic groups from tannins, proteins) could be measured.

The Malvern Master Zetasizer 3000 device was used to obtain the zeta potential, in accordance with the same procedure as in Bouaouine *et al.* (2018a). The defatted OMW materials were characterized by means of Fourier transform infrared (FTIR) spectroscopy, while the ionizable groups of defatted OMW or WSM were determined using acid-base titration (Bouaouine *et al.*, 2018a).

## 2.3 Colorimetry

The defatted OMW fraction contents, in terms of proteins (Frolund *et al.*, 1996), carbohydrates, polysaccharides (Dubois *et al.*, 1956), uronic acid (Blumenkrantz *et al.*, 1973) and polyphenols (flavonoids, tannins) (Frolund *et al.*, 1996; Salem *et al.*, 2010, respectively), were determined using colorimetric methods, with the parameters as described by Bouaouine *et al.* (2018b), and in supplementary data “table S1”.

### 2.4 Coagulation-flocculation experiments

#### 2.4.1 Synthetic water

Like in many studies of flocculation mechanisms, a synthetic water is proposed with kaolin (1 g $L^{-1}$) (Sigma Aldrich) in order to derive a turbidity (using the Hanna HI 88713 turbidimeter) of 325 ± 25 nephelometric turbidity units (NTU) (Miller *et al.*, 2008). To complete this synthetic water, humic acid (10 g $L^{-1}$) (Sigma Aldrich) (Yang *et al.*, 2010) and mineral salts were diluted (Table 1).

| | Cations (µM) | | | | Anions (µM) | | | | | pH |
|---|---|---|---|---|---|---|---|---|---|---|
| Values | $K^+$ | $Na^+$ | $Mg^{2+}$ | $Ca^{2+}$ | $Si^{4+}$ | $Cl^-$ | $HCO_3^-$ | $SO_4^{2-}$ | $NO_3^-$ | 6.5 ± 0.4 |
| | 29 | 283 | 81 | 108 | 140 | 171 | 283 | 51 | 134 | |

**Table 1:** The ionic balance of synthetic water

#### 2.4.2 Coagulation-flocculation protocol and efficient measurements

The device used for the coagulation-flocculation process was composed of six stainless steel paddles of multiple stirrers (VELP Scientifica JTL6), each containing 1 L of synthetic water. In order to determine the optimal pH, a pH variation from 3 to 12 was applied with NaOH and HCl at a constant treatment rate of 100 mg $L^{-1}$ of defatted OMW. Afterwards, a series of different doses, ranging from 0 to 160 mg $L^{-1}$ of defatted OMW, was tested to obtain the optimal treatment conditions. The sample was immediately stirred at a constant speed of 250 rpm for 10 min, followed by a slow stirring at 50 rpm for 30 min; once a 2-hour setting time had elapsed, the turbidity measurement was conducted on the supernatant.

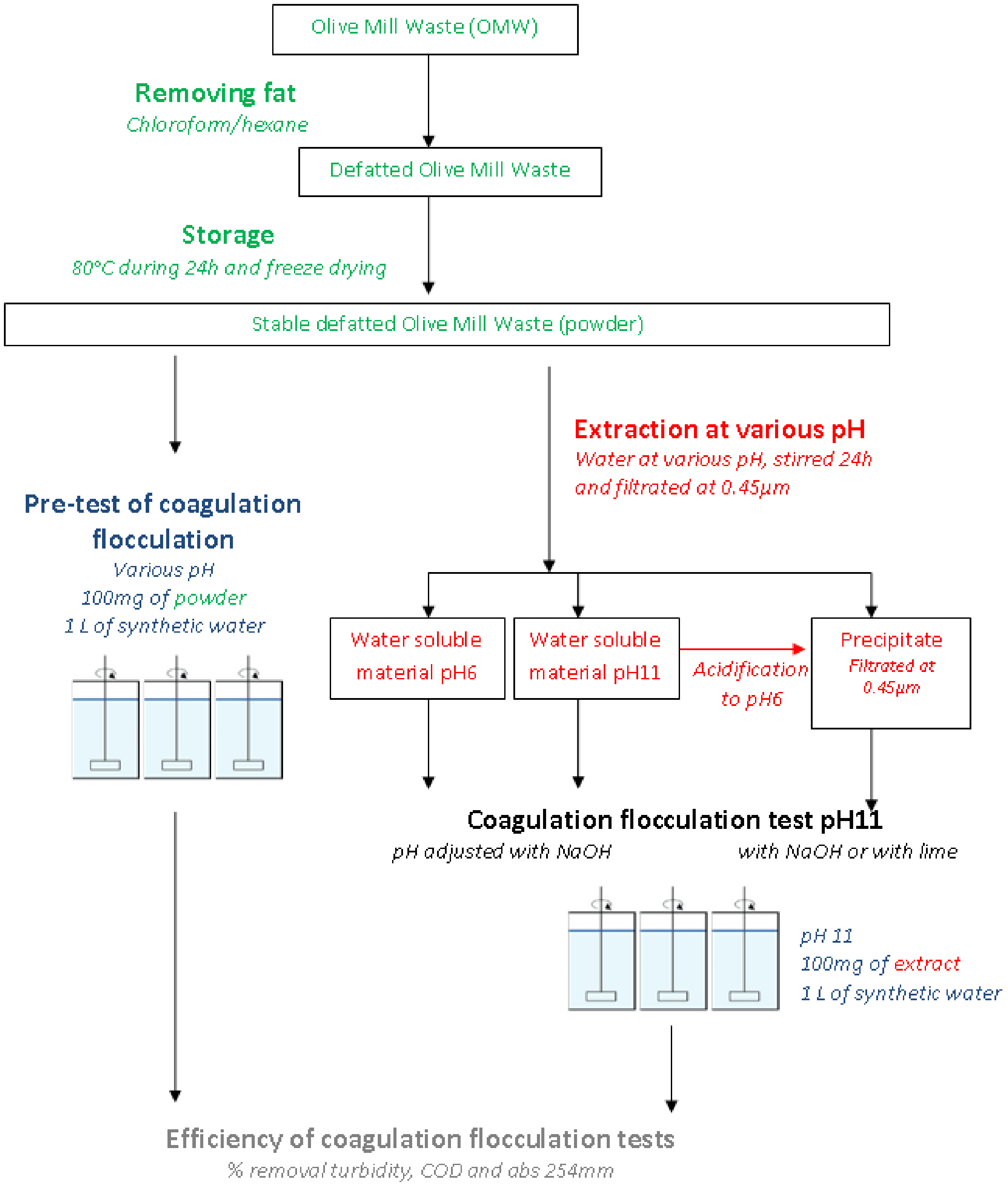


Fig. 1: Flocculent production from Olive Mill Waste and corresponding evaluation methodology

### 2.4.3 Implementation for optimization purposes

The PP treatment efficiency was compared for the case of a pH 11 adjustment by either NaOH or lime. The treatment efficiency for various PP doses was measured by both turbidity and absorbance at 254-nm. These results were then compared to a standard treatment by aluminum sulfates (AS), with a variation of AS doses from 0 to 160 mg $L^{-1}$ at a fixed pH of 7 being introduced in order to achieve optimal conditions. The three conditions described were compared for optimality.

### 2.5 Enzymatic treatment

To identify the active (macro) molecules in the defatted OMW material, hydrolytic enzymes were tested for the degradation of targeted compounds found in the defatted OMW. The enzymes and specific conditions of their actions are summarized in Table 2.

| **Enzyme** | **Hydrolyzed biochemical enzyme** | **pH** | **Temperature (°C)** |
|---|---|---|---|
| α amylase (Sigma Aldrich) | Starch | 7.0 ± 0.1 | 20 |
| Cellulase (Alfa Aesar) | Cellulose | 5.0 ± 0.1 | 50 |
| Tyrosinase (Sigma Aldrich) | Tannin | 6.5 ± 0.1 | 25 |
| Protease alcalase from *Bacillus Licheniformis* (Sigma Aldrich) | Protein | 9.0 ± 0.1 | 55 |

**Table 2**: pH and temperature enzymatic conditions for hydrolyzing precipitate (PP)

The enzymatic digestion procedure applied to the precipitate fraction was the same as that detailed by Bouaouine *et al.* (2018b). Put briefly, 18 mg of enzyme were added to a solution of 10 mL of distilled water containing an optimal PP dose of 62.5 mg $L^{-1}$ in order to reach a ratio > 10% W/W, i.e. enzyme/precipitate. The incubation period under optimal pH and temperature conditions, which depend on the specific enzyme type, lasted two hours in the previous mixture (Table 2). This preparation is then added to 1 L of synthetic water tested during the coagulation-flocculation experiment (see Section 2.4).

## 3. Results and discussion

### 3.1 Coagulation-flocculation effect

We initially sought to check the capacity of the defatted OMW material as a flocculent. The removal of turbidity at a constant dose of 100 mg $L^{-1}$, as a function of pH, is proposed in Figure 2.

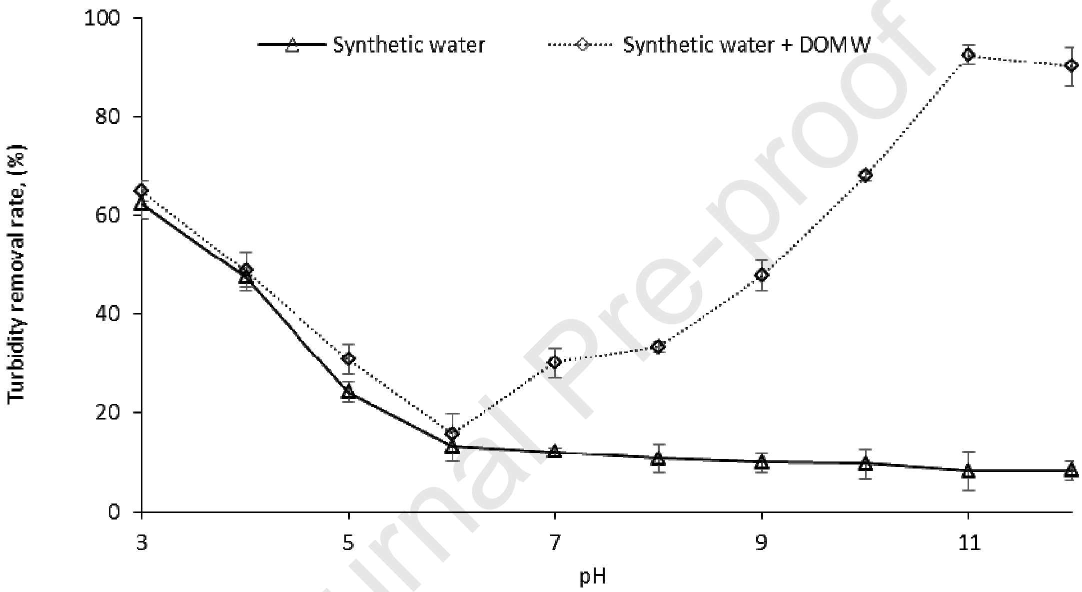


**Fig. 2:** pH effect on the coagulation-flocculation process at a fixed dose of 100 mg of defatted olive mill wastewater (OMW) material for 1 L of synthetic water; n = 3 (independent assays)

The efficiency comparison with other bioflocculants (Table 3) showed a significant reduction in turbidity with the defatted OMW at a low treatment rate. The range of pH values for optimal conditions varies (from 6 to 11) most likely depending on the extracted molecules.

| Biomaterial | Defatted OMW | *Cocos Nucifera* | *Gosspium Spp* | *Luffa cylindrica* | *Phaseolus Mungo* | Tannin |
|---|---|---|---|---|---|---|
| Effluent | Kaolin suspensions | Silica suspensions | Phosphorus wastewater | Surface water | Surface Water | Municipal wastewater |
| Initial turbidity (NTU) | 350 | 48 | - | 32 | 482 | 79 |
| Optimal pH | 11 | 8 | 6 | 9.4 | 7.6 | 8 |
| Optimal dose (mg $L^{-1}$) | 60 | 250 | 500 | 8,000 | 800 | 30 |
| Turbidity removal (%) | 93 ± 1 | 25 | > 70 | 85 | 100 | 83 |
| References | | Fatombi *et al.*, 2013 | Babayem *et al.*, 2015 | Soweyman *et al.*, 2011 | Mbogo *et al.*, 2008 | Hameed *et al.*, 2018 |

**Table 3:** Comparison of biomaterial performance

In the presence of defatted OMW, three pH value ranges can be observed. The optimal coagulation-flocculation pH equals 11.0, while the minimum is around 6. Under an acidic condition (i.e. from pH 3 to 6), turbidity removal rates do not differ significantly for synthetic water alone or in combination with defatted OMW, yet they do decrease with pH value from 64% to 15%; these results indicate the absence of any defatted OMW effect. The increase in zeta potential (i.e. reduction of negative charge), with acidification of the solution favors a coagulation mechanism of clay particles (Aljerf, 2018). High-efficiency extraction of bromocresol purple dye and heavy metals as chromium from industrial effluent by adsorption onto a modified surface of zeolite: Kinetics and equilibrium study. Journal of environmental management. 225. 120-132. 10.1016/j.jenvman.2018.07.048. along with a coagulation of colloids, in accordance with DLVO theory (see supplementary data "fig S2" which describes the zeta potential variation as pH function). Afterwards, from pH 6 to 11, when defatted

OMW is present in solution, turbidity removal reaches a peak at pH 11 with a rate of: 92% ± 2%. Lastly, from pH 11 to 12, the removal rate remains practically stable, from 92% to 90% ± 1%. The zeta potential measurement indicated negative values for both defatted OMW and synthetic water regardless of pH (between 3 and 12) (data not shown). The molecules present in defatted OMW start out reactive, while the negative charge of clay particles increases. This negative charge is unfavorable to a coagulation mechanism with a strong repellent effect between colloids. As such, the destabilization of clays and defatted OMW colloids is not a direct neutralization of particles by introduction of cationic charges (ions or colloids) in the synthetic water as shown the zeta potential measurement, but instead a flocculation, and more specifically an adsorption/bridging mechanism with a weak neutralization of clay particles but a floc structuration.

To better grasp the influence of pH, defatted OMW solubilization was performed at two pH values, i.e. pH = 11, the maximum flocculation efficiency, and pH = 6, at which flocculation appears to be minimized (Fig. 2). Moreover, in order to isolate the specific active constituents of defatted OMW materials soluble at pH 11, the pH has been subsequently adjusted to 6, so as to form a precipitate (i.e. at pH 6, component WSM 11 is inactive). The flocculant activity of the water-solubilized material at pH 6 (WSM 6) and pH 11 (WSM 11) was tested, as it was for the precipitate (PP). It is observed that both PP and WSM 11 display nearly the same efficiency, with a strong turbidity reduction, yet the effect of WSM 6 remains weak. The most robust activity of defatted OMW at pH = 11 is correlated with: i) qualitative aspects with the solubilization of specific molecules and an ionization at alkaline pH; and ii) a new conformation of macromolecules.

## 3.2 Identification of functional groups

The characteristics of the water-solubilized material at both pH values 6 (WSM 6) and 11 (WSM 11) are presented in Table 4 below.

| pH of WSM | Solubilization (RS/Defatted OMW) (%DW) | VDW (%) | $Abs_{210\ nm}$ for 1 g dry weight | $Abs_{280\ nm}$ for 1 g dry weight |
|---|---|---|---|---|
| 6.0 | 2.1 ± 0.1 | 30 ± 2 | 0.64 ± 0.01 | 0.13 ± 0.02 |
| 11.0 | 2.7 ± 0.1 | 44 ± 1 | 0.94 ± 0.03 | 0.21 ± 0.01 |

**Table 4:** Characteristics of WSM 6 and WSM 11; WSM: Water-solubilized material; DW: Dry weight; RS: Residual solid; n = 3 (independent assays)



The organic and inorganic matter contained in the defatted OMW is more readily solubilized in water at pH 11 than at pH 6 since 2.1 and 2.7 g / 100 g of matter are respectively recovered in the supernatant (factor = 1.3), thus underscoring an enrichment in organic matter (VDW increases from 30 ± 2% to 44 ± 1% for WSM 6 and WSM 11, respectively). This tendency also reflects the different nature of organic matter in the WSM fraction: note the increase in absorbance at 210 and 280 nm relative to pH, with a factor > 1.47 for the two wavelengths. At pH 11, WSM 11 exhibits higher absorbance at 210 nm, where many organic molecules can absorb (Her *et al.*, 2008). At 280 nm, only those organic molecules with an aromatic ring (or conjugated bonds), such as phenol in tannin or protein, are capable of absorption. At 280 nm, the WSM 11/WSM 6 absorbance factor ratio equals 1.61. This finding implies that WSM 11 contains more molecules enriched in aromatic groups than WSM 6.

Acid-base titrations of defatted OMW materials (Table 5) have led to detecting four acidic constants ($pka_s$): $pKa_1$ (2.5 ± 0.2) corresponds to the carboxyl group from free amino acids (Vcélakova *et al.*, 2004); $pKa_2$ (6.5 ± 0.3) is mainly correlated with -COOH from polysaccharides and secondary alcohols (Droussi *et al.*, 2009); $pKa_3$ (9.0 ± 0.4) may be associated with the phenol groups (Ragnar *et al.*, 2000) from either lignin (Beliy *et al.*, 2010) or tannin, e.g. catechin (Martinez *et al.*, 2005); and lastly $pKa_4$ (11-12) could correspond to amino acids like tyrosine (Sjoholm *et al.*, 1974) or else to free sugars, such as glucose, arabinose and fructose (Bhattacharyya and Rohrer, 2012).

| $pKa_s$ | Defatted OMW | WSM 11 | WSM 6 | PP |
|---|---|---|---|---|
| $pKa_1$ | 2.5 ± 0.2 | | | |
| $pKa_2$ | 6.5 ± 0.3 | | 6.5 ± 0.3 | |
| $pKa_3$ | 9.0 ± 0.4 | 9.0 ± 0.3 | | 9.0 ± 0.4 |
| $pKa_4$ | 12.0 ± 0.7 | 11.8 ± 0.4 | 11.0 ± 0.8 | 12.0 ± 0.5 |

**Table 5:** $pKa_s$ values for all defatted olive mill wastewater (Defatted OMW) used as a raw material, water-solubilized material (WSM) at pH values of 6 and 11 and precipitate (PP); n=2

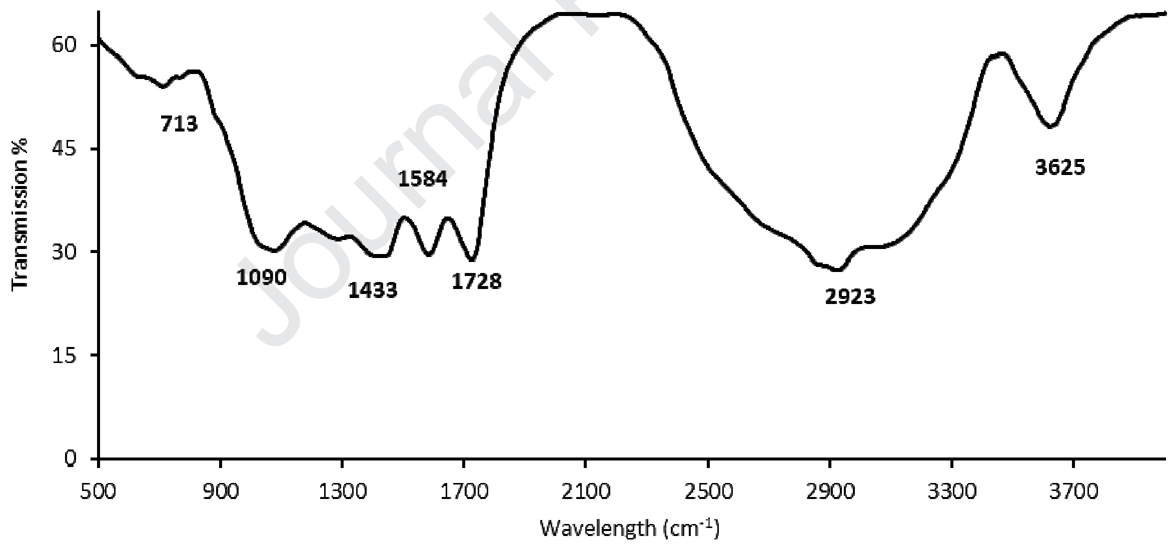


**Fig. 3:** FTIR spectrum of defatted olive mill wastewater (Defatted OMW) material

According to the literature, a comparison drawn between the results detected in FTIR bands and acidic constants of ionizable groups has revealed that $pKa_1$, which corresponds to carboxyl groups (Vcélakova *et al.*, 2004), as confirmed by the appearance of a peak at 1,100 $cm^{-1}$ (Bouatay *et al.*, 2014), might possibly be associated with tryptophan. The presence of -

COOH groups has already been proven by both $pKa_2$ and the band at 710 $cm^{-1}$ (Droussi *et al.*, 2009), which may be corroborated by the uronic acid (polysaccharides) (Elhajjouji *et al.*, 2007). pKa3 is correlated with phenol groups (Droussi *et al.*, 2009), and this presence may pertain to the tannins and/or lignin. Lastly, $pKa_4$, as represented by -C=O groups (Elhajjouji *et al.*, 2007) and having appeared in FTIR by the peak absorption at 1,728 $cm^{-1}$ (Moran *et al.*, 2008), could correspond to polysaccharides (Fig. 3).

The defatted olive mill wastewater (OMW) material displays four acidic constants, whereas WSM 6, WSM 11 and PP only display two $pKa_s$. $pKa_2$, which represents uronic acids (Wang et al, 1991), is observed to be common between defatted OMW and WSM 6 materials (except for the fact that the removal efficiency of WSM 6 is only 23% with 325 and 250 NTU for turbidity in and out respectively).

$pKa_3$, which corresponds to phenol groups, and $pKa_4$ may however align with sugars and proteins, with common features across the defatted OMW material, WSM 11 and PP. From these observations, polyphenols, proteins and sugars are all suspected to be correlated with turbidity removal efficiency as reported in the literature (Bouaouine *et al.*, 2018a, Fatombi *et al.*, 2013, and Shamsjenati *et al.*, 2015, respectively)

### 3.3 Identification of reactive molecules

In order to identify the major constituents involved in the flocculation reaction, we have determined by means of colorimetric assays the main biochemical families, i.e. proteins, polysaccharides, uronic acid, phenol and tannins included in phenol family, for the defatted olive mill wastewater (OMW) materials plus the three preparations (WSM 6, WSM 11 and PP). Table 6 presents the contents of biochemical molecules on WSM 6, 11, PP and their WSM 11 / WSM 6 and PP / WSM 11 ratios.

| Sample | Protein (SAB eq.) | Polysaccharide (glucose eq.) | Uronic acid (gluc. acid eq.) | Phenol (gallic acid eq.) | Tannin (catechin eq.) |
|---|---|---|---|---|---|
| WSM 6 ($g.g^{-1}$) | 0.145 ± 0.001 | 0.028 ± 0.001 | 0.29± 0.01 | 0.165 ± 0.001 | 0.026 ± 0.001 |
| WSM 11 ($g.g^{-1}$) | 0.152 ± 0.001 | 0.023 ± 0.001 | 0.24 ± 0.01 | 0.17 ± 0.01 | 0.028 ± 0.001 |
| PP ($g.g^{-1}$) | 0.15 ± 0.01 | 0.023 ± 0.001 | 0.24 ± 0.01 | 0.175 ± 0.001 | 0.05 ± 0.01 |
| WSM11/WSM 6 | 1.05 ± 0.01 | 0.82 ± 0.02 | 0.83 ± 0.02 | 1.03 ± 0.01 | 1.33 ± 0.02 |
| PP/WSM 11 | 1.00 ± 0.02 | 1.00 ± 0.01 | 1.00 ± 0.02 | 1.03 ± 0.01 | 1.55 ± 0.01 |

**Table 6:** Contents of biochemical molecules on WSM 6, 11, PP and their ratios; n = 3 (independent assays)

Table 6 shows that the [WSM 11 / WSM 6] ratio for protein and phenol nearly exceed 1. For tannin, the [WSM 11 / WSM 6] ratio is greater than 1 (1.33), and this same trend was observed with the ratios between PP and WSM 11 (1.55). The phenol/polyphenol family includes tannins and lignin, which indicates that tannins are particularly well solubilized at pH 11 and concentrated in PP, as compared to other biochemical families. These results confirm that phenol groups (see Table 5) and therefore tannins could be among the most active molecules in the flocculation process and soluble at pH 11.

In order to specify the biochemical group for tannins, which includes condensed tannins and flavonoids, the condensed tannins and total flavonoids for WSM 6, WSM 11 and PP were determined (Table 7). The increase in total tannin concentrations between WSM 6 and WSM 11 (factor = 1.33) and between WSM 11 and PP (factor = 1.55) confirms the specific solubilization in alkaline pH and the concentration by precipitation, respectively. For condensed tannins, an increase was also found in concentrations between WSM 6 and WSM 11 (factor = 1.3) and between WSM 11 and PP (factor = 1.2). This same trend could also be observed for total flavonoids, with a strong increase from WSM 6 to WSM 11 (factor > 1.3), hence an increase between WSM 11 and PP (factor > 2.1).

| Sample | Tannins, in mg.g$^{-1}$ of DW (catechin eq.) | Condensed tannins, in mg.g$^{-1}$ of DW (catechin eq.) | Flavonoids, in mg.g$^{-1}$ of DW (catechin eq.) |
|---|---|---|---|
| WSM 6 | 30 ± 1 | 20 ± 2 | 12 ± 2 |
| WSM 11 | 40 ± 1 | 26 ± 1 | 16 ± 3 |
| Precipitate (PP) | 62 ± 1 | 31 ± 1 | 34 ± 2 |

**Table 7:** Contents of specific polyphenols: total tannins, condensed tannins and flavonoids from solubilized defatted olive mill wastewater (OMW), as determined by colorimetric methods; DW: dry weight; n=3

These results reveal that the molecules responsible for the flocculation mechanism belong to flavonoids and/or condensed tannins. Hameed *et al.* (2018) found the reactivity of commercial tannin for turbidity removal from a municipal wastewater at a rate of 83% for a of 30 mg L$^{-1}$ treatment rate and a pH value around 8. Moreover, Ozacar *et al.* (2002) showed that at pH 11, tannins extracted from Valonia were highly efficient, with an 80.5% decrease in turbidity for a synthetic surface water at a dose of 100 mg L$^{-1}$. Other authors have demonstrated that polyphenols (Jeon *et al.*, 2009), proteins (Ndabigenegesere *et al.*, 1998) and polysaccharides (Miller *et al.*, 2008) represent the active molecules in grape seed, *Moringa Oleifera* and cactus *Opuntia ficus indica*, respectively. For these reasons, other tests are essential in confirming the potential of proteins and polysaccharides (according to the results in Table 6 and Fig. 3) to be the constituents responsible for the flocculation mechanism by defatted OMW.

To complete the investigation of flocculent biochemical molecules present in PP, this fraction was processed with four hydrolytic enzymes (Table 2): α amylase, cellulase, protease alcalase, and tyrosinase. If active molecules are being hydrolyzed, then the modified PP shows a lower efficiency for the flocculation of synthetic water. To validate these results, two controls were performed, i.e.: Control 1 (preparation with enzyme alone), and Control 2 (preparation without any enzymes, i.e. PP alone). The four Controls 1 and 2 indicated a turbidity removal efficiency of 15% ± 1% (turbidity in 325 NTU and turbidity out 275 NTU) and 83% ± 1% (turbidity in 325 NTU and turbidity out 55 NTU), respectively.

Flocculation efficiency after degradation by enzymes exhibits hardly any effect for α amylase and protease alcalase, with 79% ± 2% and 80% ± 3% turbidity removal rates, respectively. With cellulose however, the turbidity removal by coagulation-flocculation was decreased to 60% ± 2% and to an even greater extent with tyrosinase: 24% ± 4%. These results reveal that enzymatic treatments are in agreement with colorimetric dosages (Fig. 3 and Table 6), FTIR and acid-base titrations, thus confirming that tannins (condensed tannins and/or flavonoids) and cellulose, present in defatted OMW materials (Hamdi *et al.*, 1993), do constitute the active groups responsible for the flocculation phenomenon by defatted OMW. Data from the literature confirm the efficiency of cellulose from cotton pulp in coagulation-flocculation (Song *et al.*, 2010). These results thus suggest that tannin and/or flavonoid may act as a coagulant by destabilizing colloids by adsorption on cationic sites (iron oxides, aluminum or calcium) may be present on the clays surface (Xu *et al.*, 1988) . Septian *et al*. (2018) showed that sulfadiazine adsorption was possible onto montmorillonite and kaolinite at pH 8. A mechanism of p-p interaction between clay surface and organic functional groups was observed for polycyclic aromatic hydrocarbons (Yanfen *et al*., 2018). Binding is attributed to p-p interaction between an oxygen plane on the clay and aromatic ring on the molecule. Following coagulation, the cellulose interacts with polyphenol (Aldred *et al.*, 2009) and may form a large cross-linked polymeric network responsible for flocculation by means of bridging.

### 3.4 The mechanism involved and highlights

The strong negative charge of colloids (insoluble material of defatted OMW or bentonite) under an alkaline condition is unfavorable to a coagulation mechanism with strong repellent effects. In accordance with DLVO theory, the aggregation of colloidal aqueous dispersions is improbable when the energy barrier is maximum. Particles remain dispersed throughout the medium. At larger distances the energy profile became minimum and chemical flocculation is possible by polymer bridging. A model of chemical interactions between active molecules from PP and kaolinite is proposed in Figure 4. At pH 11, tannins are ionized and could establish ionic bonds with the Al of kaolinite (Kuntic *et al.*, 2003). Cellulose can be adsorbed on kaolinite by hydrogen bonding and contribute to floc structuration and both flocculation by bridging and sweeping coagulation (Sjoberg et al 1999). Adsorption on the basal surface of kaolinite occurs mainly on the aluminum hydroxide surface and at the edge; polyphenol may be linked by hydrogen bonds to aluminol or silanol groups and principally to aluminol in alkaline media (Bergaya and Lagaly, 2013). To explain the interaction taking place between polyphenols (tannins) and cellulose, two means merit discussion: i) hydrogen bonds, and ii) hydrophobic interactions (Mateus *et al.*, 2004).

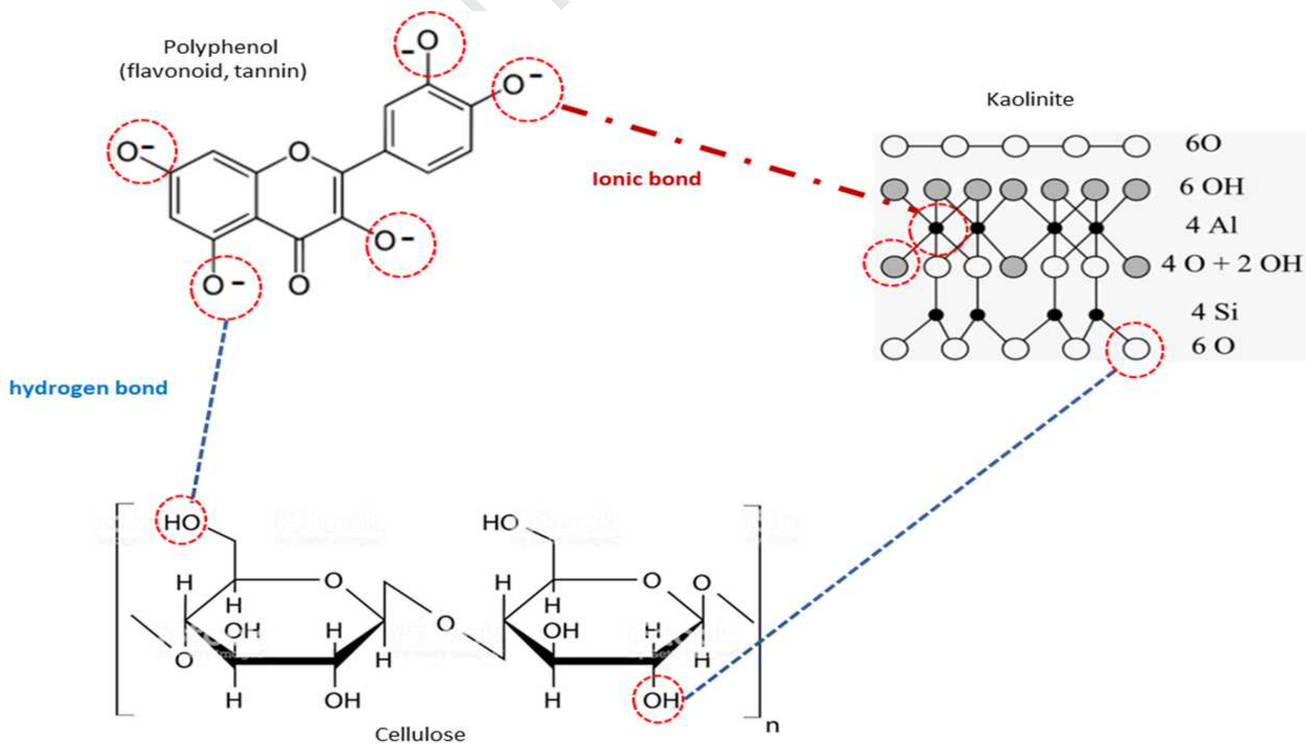


**Fig. 4:** Chemical interactions involved in coagulation-flocculation between quercetin, cellulose and kaolinite

Coagulation-flocculation reactions with defatted OMW material (Fig. 2) are indicative of the potential in substituting chemical coagulants/flocculants. Additional research has been proposed to experiment on integrating the most active material of defatted OMW (PP) in a water treatment process. The treatment with precipitate adjusted to pH 11 with NaOH or lime is compared to a standard reference for wastewater treatment, aluminum sulfate (AS) (Table 8).

| | Dose (mg $L^{-1}$) | COD (mg $L^{-1}$) | | Turbidity (NTU) | |
|---|---|---|---|---|---|
| | | in | out | in | out |
| $Al_2(SO_4)_3$ (pH = 7) | 80 | 14.8 | 0.7 ± 0.3 | 325 | 7 ± 3 |
| Precipitate (pH = 11 with NaOH) | 60 | 14.8 | 21.6 ± 1 | 325 | 21 ± 3 |
| Precipitate (pH = 11 with lime) | 60 | 14.8 | 7.4 ± 0.3 | 325 | 23 ± 3 |

**Table 8:** Comparison of the efficiency treatment between aluminum sulfate (AS) and precipitate (PP) with a pH adjustment by NaOH or lime; n=3 (independent assays)



Absorbance of the synthetic water at 254 nm, which corresponds to aromatic groups from humic substances, equals 1.2 ± 0.2.

Table 8 presents the optimal dose for the three treatments, the residual COD and turbidity under these conditions. The best efficiency with AS was observed at 80 mg $L^{-1}$, with 95 ± 2% and 98 ± 1% of absorbance and turbidity drops, respectively. In parallel, the use of PP has demonstrated competitive results, as adjusting pH with NaOH led to turbidity removal rate of 83 ± 1%, albeit with an increase in absorbance at 254 nm (-46 ± 1%) for a treatment rate of 60 mg $L^{-1}$. In contrast, pH adjustment by lime has resulted in a higher removal of turbidity (93 +

1%) and an improved absorbance removal at 50 ± 2% for a dose of 60 mg $L^{-1}$. Lime exerts an effective action on the coagulation-flocculation mechanism (Rharrabati *et al.*, 2018). The divalent cation $Ca^{2+}$ contributes to interparticle bridging between all anionic species: kaolinite, polyphenols, but also the cellulose or humic acids of synthetic water (Choudhary *et al.*, 2019). This preparation from defatted OMW (precipitate fraction (PP) obtained after decreasing pH to 6 upon extracting the alkaline water), used in conjunction with a lime-based pH regulation (pH = 11), yields adequate turbidity and abs 250-nm removal rates. This preparation from vegetal waste appears to be a possible alternative to water treatment (natural water or wastewater) by metal salts (iron or aluminum) or synthetic polyelectrolytes, both of which may be toxic.

## 4. Conclusion

Olive mill wastewater (OMW) was tested as a bioflocculant in the coagulation-flocculation process for water treatment. After OMW removal steps, a high removal rate (92 ± 1%) of the synthetic water turbidity was obtained at pH 11 with a treatment dose of 100 mg $L^{-1}$. The active part of this material can be isolated by solubilization under alkaline conditions and precipitation by means of neutralization (PP). Enzymatic treatment, colorimetric dosage, FTIR and acid-base titration all confirm that tannins and/or flavonoids and cellulose constitute the active groups responsible for the flocculation phenomenon. PP exhibits a high performance in turbidity removal, compared with other vegetal flocculants. Moreover, the adjustment of pH using lime, associated with a treatment rate of 60 mg $L^{-1}$ of PP, has allowed optimizing the process with removal rates of 93 ± 1% and 50 ± 2% for turbidity and absorbance at 254 nm, respectively. This study has demonstrated that olive mill wastewater may be reliably reused for water treatment in a flocculation process.

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**Highlights**

1) Defatted olive mill wastewater (DOMW) could be a bioflocculant for water treatment
2) Two biochemical families represent the active constituents: tannins and cellulose
3) Good efficiency compared to other green flocculant
4) Comparable turbidity removal with $Al_2SO_4$ was observed
5) PH adjustment with lime improve the efficiency