Study of Physico-chemical and Microbiological Combined Treatment of Olive Oil Mill Wastewater

*Elharchli ELhassan, *Ghomari Oumaima, **Chokrafi Fatima-Zohra,*Chraibi Marwa,**Khalil Fouad

Abstract—The OMWW (olive oil mill wastewater) is the main liquid from olive oil mills. By its chemical composition, it has a very high polluting power. This products stemming from the triturating of olives very rich in organic matters (consisted phenolic, lipids) is often spread as is in the nature without controlled way, so exposing the aquatic ecosystems, has an inevitable pollution and degrades the quality of the circles. Our study is interested in the treatment of OMWW by combination of two physico-chemical and microbiological processes. The first treatment consists on a coagulation-flocculation revealing a decrease of the turbidity, a sludge production and an important discoloration of OMWW. The rate of phenolic compounds dejection after coagulation-flocculation using the ferric chloride and the aluminum sulfate are respectively 54 % and 64 %. The second microbiological treatment bringing in the capacities of A. Niger, C. albicans and C.tropicalis to reduce the rates of the phenolic compounds respectively of 100 %, 56 % and 78 % for diluted OMWW and also with 54 %, 27 % and 33 % for the raw OMWW. The decrease of the antibacterial activity of OMWW after the treatment by coagulation-flocculation is bound to a reduction of phenolic compounds by this process.

Index terms— OMWW, coagulation, flocculation, biological treatment, mushrooms yeast, polyphenols, antibacterial activity.

1 Introduction

Olive oil is a typical product of some countries situated on the north and south banks of the Mediterranean Sea and present 94 % of the world production [1]. Morocco was always an important producer of olive oil, with a continuous tendency to the increase of the levels of production [2]. Olive oil mill wastewater (OMWW) is waters of vegetation which are generated during the extraction of the olive oil. Receiving waters of OMWW become strongly charged in organic matters and in pollutants and lose their capacity to auto-purify. OMWW represents one of the most contaminating effluents among those produced by the agro-food industries [3]. The criteria of pollution of OMWW limit themselves to three main factors: The acidity, the high conductivity due to the addition of the salt during the storage of olives before their triturating and the concentration raised in organic matter represented essentially by the phenolic compounds (P.C) which are responsible for the toxicity and for the brown-reddish tint to black of OMWW

*Laboratoire de Biotechnologie Microbienne. Faculté des Sciences et Techniques, Route d'Imouzzer 30000 Fès. Maroc.
**Laboratoire de Chimie Appliquée, Faculté des Sciences et Techniques, Route d'Imouzzer 30000 Fès. Maroc.
Université Sidi Mohamed Ben Abdellah, Fès. Maroc.
Corresponding author : Elharchli Elhassan
hassanelharchli@gmail.com

Therefore, the rejection of these effluents in rivers and sewers without any preliminary treatment presents a negative impact on the environment because OMWW contains toxic P.C which cause the pollution of superficial and underground waters, and which have antimicrobial effects towards the microbial communities of the ground and also powers phyto-toxicity [4,5]. OMWW also peaks nitrogenous compounds, sugars, organic acids, and pectins what increases their organic load. So, the variability of the physico-chemical characteristics of OMWW is a function of weather conditions, of cultures of olives, of the degree of fruits maturation, of the duration of storage and finally of the extraction process [6]. The high polluting activity of OMWW is linked to low pH, high tells of mineral salts, and wide amounts of organic molecules, especially polyphenolic mixtures with different to molecular weights [7]. These considerations led several researchers to opt for the adequate way of the treatment and the valuation of OMWW to limit their pollution [8,9]. Conventional methods for the removal of phenolic compounds from OMWW can be divided into three main categories: biological [10,11,12], chemical [13,14,15] and physical treatment [16,17,18]. Until now various studies of OMWW treatments solve only partially the problem and most of the proposed processes remain insufficient. The physical treatment allows only a simple separation of the water from suspension materials, what engenders the appearance of muds at the end of treatment and the management of which is not rational completely. The chemical processing of OMWW asks for big quantities of reactive, because of the wealth in organic and mineral matters. The aerobic classic biological treatment requires strong dilutions so that the process is not inhibited by the toxic phenolic compounds. Furthermore, this aerobic system asks for a very important energy for the aeration and the admixture of the environment. The anaerobic biological processing is limited by problems of acidification, toxicity and biodegradability. OMWW cannot be thus handled by a simple physical, chemical or biological process. A series of treatments turns out to be necessary to reduce the strong concentration to P.C. Our study joins in a vision of Combined Treatment of OMWW. We then led two combined processes by treatment of the OMWW from "Hamria" region in Fez (Morocco). The first physico-chemical treatment consists in coagulations-flocculation of OMWW while the second biological treatment brings in the potentialities of the mushrooms (*Aspergillus* and *Candida)* to degrade the P.C. We finalized in parallel a physic-chemical and micro-biological characterization of these OMWW and we made out a will the antibacterial effects that show their P.C.

2 Materials and Methods

2.1 Sampling

The samples of the OMWW which served during our study are taken of a triturating unity of olives from "Hamria" region in Fez (Morocco). The takings of OMWW are made during the campaign on 2014-2015. Samples were taken from the storage pond. Analyses are realized dice reception in the laboratory.

2.2 Physico-chemical characterization

The measures of the pH and the electric conductivity (E .C) are respectively made by a typical pH-meter (HANNA instruments model 210) and a conduct-meter of type (inoLabLevel 1). The turbidity is measured by means of a turbid-meter of model type AQUA LYTIC AL250T-IR. The intensity of the color is determined by the measure of the absorbance meanwhile of the visible [430nm-710nm] using a visible spectrophotometer (JENWAY model 6051).

2.3. Microbiological characterization

The microbiological analysis of samples is realized from reception in the laboratory. After homogenization of OMWW, a series of dilutions in some sterile distilled water is realized. A volume of 0,1 ml of every dilution is displayed over Petri dishes containing cultural mediums. We used the LB, YPG and EM mediums for the culture respectively of bacteria, yeasts and mushrooms. The incubation of the displayed boxes is made in 37°C during 24 hours for bacteria and in 30°C during 48 hours at 72 hours for yeasts and mushrooms. The YPG and EM mediums are added to Kanamycine to inhibit the bacterial growth.

2.4. Physico-chemical treatment

The coagulation-flocculation tests are realized by using a flocculate with helixes (jar-test). This device allows shaking simultaneously the liquid contained in a series of beakers each of 400 ml with OMWW. This series containing the same samples of OMWW is subjected to increasing doses of the coagulant to determine the optimal dose. The mixture is shaken in spin-dried (200 rpm /10 min). The speed is afterward reduced to 45 lasting rpm /30 min. After 180 minutes of settling, the volume of muds is measured then the floating is got back to make the analyses of the physico-chemical parameters. The optimal concentration of the coagulant is determined on the basis of the turbidity, of the produced muds volume and of the visual aspect of floating.

2.5. Microbiological treatment

Two yeasts (*Candida albicans* and *Candida tropicalis*) and a mushroom (*Aspergillus niger*) are used for the microbiological treatment of OMWW. 20 ml of various phases of OMWW treatments (raw OMWW, diluted OMWW, OMWW before and after physico-chemical and microbiological treatments) are sterilized in a temperature of 120°C during 20 minutes, then we added 1ml of the microbial suspension. After incubation in 30°C under agitation during 48 hours. Sample of 5 ml is taken and spin-dried (5000rpm). Floating got back was the object of phenolic compounds dosage.

2.6 Determination of PC

2.6.1 Preparation of phenolic extracts

Extraction of P.C from OMWW is a liquid-liquid type extraction which is based on the principle of solubility in organic solvents. The choice of solvent depends on the nature of compounds to extract, their solubility in the solvent and the nature of the plant material. Phenolic compounds contained in OMWW are extracted according to the method of MACRO [19]. OMWW have prior undergoes pre- treatment by hexane to remove lipids. It is a liquid-liquid ethyl acetate extraction in often used for this type of extraction Della Grace [20]. Ethyl acetate is added to the delipidated OMWW (V/V). The whole is homogenized, after centrifugation at 5000 rpm / 12 min; the mixture is completely separate in two phases: the supernatant containing rich in polyphenols ethyl acetate and the pellet with the rest of OMWW. The supernatant P.C rich suffered evaporation under vacuum in a rotary evaporator at 75°C. These phenolic extracts have been used for the determination of the P.C. and antibacterial activity test.

2.6.2 Determination of phenolic extracts

The P.C. are determined by the Spectro-photometric method [21]. 5 ml of the diluted extract (1 ml of extract phenolic + 4ml distilled water) is added to 500 µl of distilled water, 500 µl of the solution of sodium carbonate (Na_2CO_3) and 500 µl of the Folin-Ciocalteu reagent. After 30 min of incubation at 40°C and in the dark, the absorbance is measured at spectrophotometer (765 nm). Quantification of phenolic compounds was made on the basis of alinear (y = a x) calibration curve carried out using Gallic acid as reference. The results will be so expressed in Gallic acid equivalents.

2.7 Testing of antibacterial activities of OMWW

The antimicrobial activity is established by using the method of diffusion on agar. The bacterial strains selected for these tests are derived from microbial biotechnology laboratory. It is *Escherichia coli*, *Salmonella*, *Pseudomonas aeroginosa*, *Staphylococcus aureus*, *Bacillus cereus* and *Bacillus subtilis*. After incubation of Petri dishes at 37°C for 24 hours. The inhibition aureoles diameters are measured. The experiments are repeated twice for each test.

3 RESULTS AND DISCUSSION

3.1 Physical and chemical characterization of OMWW.

Table 1 shows the measured physic-chemical characteristics values of OMWW studied. We then noticed an acid pH (4,5). This acid character makes it very difficult for the biological treatment of OMWW gross having regard to the conditions for development of micro-organisms [22].The high electrical conductivity and salinity values which are respectively 7 $mS.cm^{-1}$ and 4,7 are owed to salting practiced to keep olives up to triturating. This OMWW introduces turbidity in the order of 667 NTU due to the high contents of subjects in suspension and organic substances. These rejections are also characterized by the predominance of P.C (4,3 g/l) which confer on them an antimicrobic power. The high concentration of PC could limit any natural biodeterioration, and as a result could cause a deep disruption of Acceptor ecosystems of OMWW.

Table 1: Physico-chemical parameters of OMWW

OMWW raw characteristics	Values
pH	4,5
C.E ($ms.cm^{-1}$)	7
Salinity	4,7
Turbidity (NTU)	667
P.C (g/l)	4,3

3.2 Microbiological characterization of OMWW

The bringing under cultivation of micro-organisms coming from OMWW revealed a microbial load characterized particularly by a wealth in yeast and in other fungi. Counts of the different colonies appeared in all dilutions were used to estimate the biomass of yeast and fungi which is respectively of the order of 10^6 $UFC.ml^{-1}$ and 6 10^6 $UFC.ml^{-1}$ (table 2). The Petri dishes intended for the growth of bacteria did not demonstrate colonies. These data compound with those got by the work of [21] that showed that in OMWW, fungi and yeasts are able to grow more than bacteria. This is related to the physico-chemical characteristics of OMWW that inhibit bacterial growth including the presence of P.C.

Table 2: Microbiological characterization of OMWW

Micro-organisms	UFC/ml
Mushrooms	$6\ 10^6$
Yeats	$4\ 10^6$
Bacterias	0

3.3 Physicochemical treatment of OMWW

This treatment of OMWW by physicochemical method coagulation-flocculation aims on one hand, the comparison of the effectiveness of this technique, in comparison with that of the microbiological way. In other parts, the combination of these two treatments for a better action on the depression of PC of OMWW.

3.3.1 Treatment with ferric chloride

The conditions and the results of the tests of coagulation using ferric chloride as a coagulant are shown in table 3. All tests were conducted with an adjustment of pH around 7. Indeed, this area is within the optimum range of pH for ferric chloride. Note that the progressive addition of ferric chloride to the OMWW causes a decrease in pH from 4.9 to 1.19. The volume of mud was measured according to the time of decantation (8 hours).

Table 3: Conditions and results of the tests to $FeCl_3$

Test	1	2	3	4	5
Quantity of coagulant (g/l)	10	12.5	15	17.5	20
pH after addition of coagulant	1.08	1.19	1.10	1.06	1
adjusted pH	7.63	7.66	6.58	6.75	7.40
Volume of mud (Ml)	150	230	220	220	210
Turbidity (NTU)	70.5	51	53.4	56.3	61

Figure 1 show the evolution of turbidity and the volume of sludge decanted the coagulant dose-dependent parsing to $FeCl_3$. It noted that turbidity is minimal for the amount of 12.5 g/l of $FeCl_3$ while the volume of sludge is maximum around this same dose and decreases with the addition of the coagulant. It gets a discoloration of OMWW for doses from 12, 5 g/l of $FeCl_3$. It can be concluded that optimal dose corresponds to 12.5 g/l of $FeCl_3$ because it allows a good reduction of turbidity (heading 90.14%) and a good discoloration

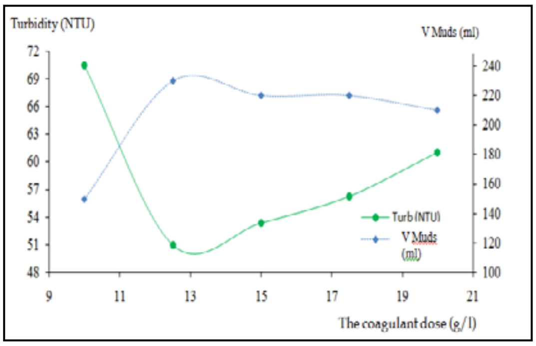

Figure1: Variation of turbidity and of volume of muds according to the dose of $FeCl_3$

3.3.2 Treatment with aluminum sulfate

The conditions and the results of the tests of coagulation using aluminum sulfate are shown in table 4.

Table 4: Conditions and results of the tests to $Al_2(SO_4)_3$

Test	1	2	3	4	5
Quantity of coagulant (g/l)	15	17.5	20	22.5	25
pH after addition of coagulant	3.43	3.4	3.4	3.4	3.34
adjusted pH	6.61	6.56	6.59	6.65	6.55
Volume of mud (Ml)	190	205	210	295	240
Turbidity (NTU)	39.2	38	34.4	32.2	33

Figure 2 shows the evolution of turbidity and the volume of sludge decanted depending on the dose of coagulation (aluminum sulfate). The pH is adjusted to a value of 6.50. As in the case of ferric chloride, and after observation of the evolution of the various parameters, we concluded that the dose of 22.5 g/l of $Al_2(SO_4)_3$ allows the best reduction of turbidity (96.81%) and a good bleaching. The maximum of sludge produced are of 737.5 (ml/l) of OMWW diluted.

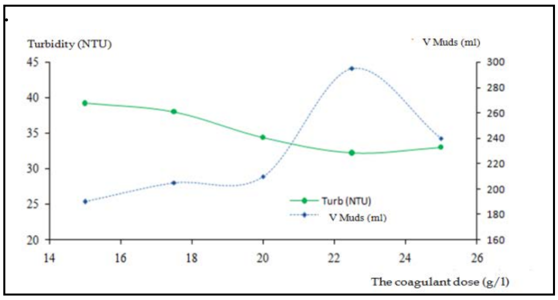

Figure2: Variation of turbidity and of volume of muds according to the dose of $Al_2(SO_4)_3$

3.3.3 Comparison of the results of the two coagulants

Table 5 includes the corresponding results the optimal area of testing with both types of

coagulants (aluminum sulfate and ferric chloride). Aluminum sulfate allows a good reduction of turbidity (96.81%) with a maximum volume of sludge. On the other hand, the chloride ferric appears less effective (heading 90.14% of removal of turbidity) but produces less sludge.
Figure 3 shows the change in the absorbance of OMWW treated by ferric chloride and aluminum sulfate in an interval of 430 nm a 710 nm wavelength. Results showed that the two coagulants allow a good reduction in absorbance with better efficiency for aluminum sulphate.

Table 5: Turbidity and volume of mud of the OMWW treated by different coagulants

Coagulant	Chlorure ferrique	Sulfate d'aluminium
Quantity of coagulant (g/l)	12.5	22.5
Volume of mud (ml)	230	295
Turbidity (NTU)	51	32.2

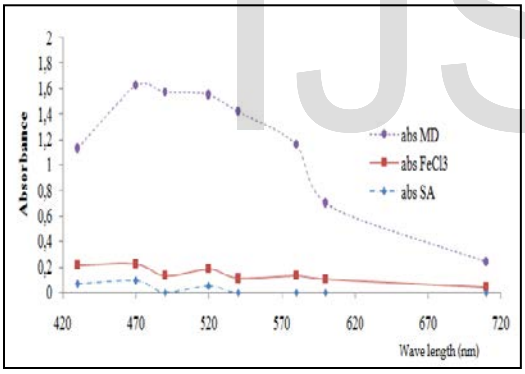

Figure3: change in absorbance of OMWW in the interval [430 nm - 710nm]

3.3.4 Abatement rate of P.C by physico-chemical treatment.

Figure 4 shows the rate of reduction of PC after chemical treatment. The results show that he has a significant decrease of phenolic compounds. This would be attributed to the phenomenon of adsorption of Colloids on the formed flocks

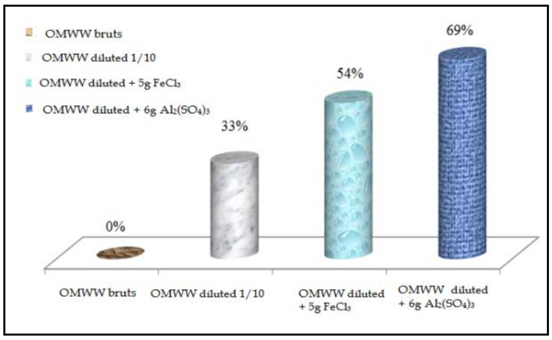

Figure 4: Rate of depressions in C.P before and after treatments physicochemical of OMWW

3.4. Microbiological treatment of OMWW

The microbiological treatment of OMWWs by three microorganisms demonstrated a changeability of the rates of depression of C.P according to studied conditions (face 5). We noted that the treatment of the raw OMWW shows depressions which exceed 54 % for *A. niger*. While for diluted OMWW the reduction of P.C is 100 %, 56 % and 78 % with the treatment respectively by *A. niger*, *C. albicans* and *C.tropicalis*. This suggests that OMWW concentrated would be responsible for the decrease in the activity of microorganisms. This decrease is related to the nature of OMWW strongly loaded in organic pollutants, in particular the large concentrations of toxic compounds such as tannins and the P.C. Some of these compounds could have an antimicrobial effect which manifests itself by the impairment of the cellular membranes [23]. In more, P.C in high concentration may focus on enzymes whose activity would be affected and microbial cells would be deprived of intermediate metabolites, which succeed in the inhibition of their growth. Results also show that the capacity of *A. niger* to assimilate P.C that of other microorganisms in different types of OMWW is higher than. Indeed, the chitin of the walls of *A. niger* is known by the capacity to fix P.C, what can contribute to their elimination of OMWW [24]. *A niger* is capable at the same time of degrading aromatic polymers as tannins and anthocyans and aromatic monomers [64]. On top of that, the catéchol who is an intermediary importing the aerobic catabolism of P.C, is easily degradable by *A. niger* thanks to his catechol 1,2-dioxygénase [25]. *Candida tropicalis* also has the capacity to eliminate 33 %, 78 %, 65 %, 31 % P.C.for the raw, OMWW, treated by 5g of $Fecl_3$ and 6g as $A1_2(SO_4)_3$ respectively. These results are superior to those got after the treatment of

OMWW by *Candida* albicans (27 %, 56 %, 57 %, 2 % for the raw OMWW, treated by 5g of $Fecl_3$ and 6g $A1_2$ $(SO_4)_3$ respectively). But the treatment of OMWW by this two type Yeast remains limited since they are considered to be pathogenic strains.

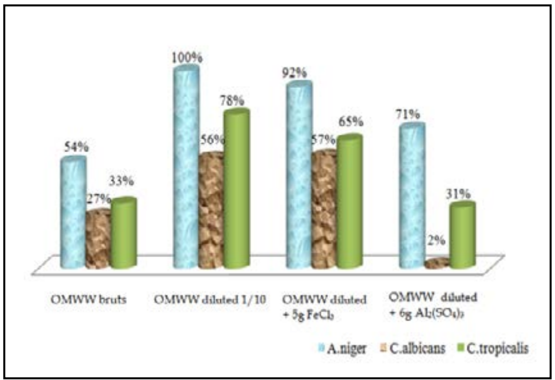

Figure 5: Rate of depressions in C.P of OMWW treated by the three fungal strains

Mushrooms are generally more effective than the yeast in the treatment of OMWW. This is probably due to their more efficient enzyme system in the degradation of the P.C. compared yeasts. We were also able to conclude from figures 4 and 5 that the physico-chemical treatment of a biological treatment allows to increase the rate of reduction of the P.C.

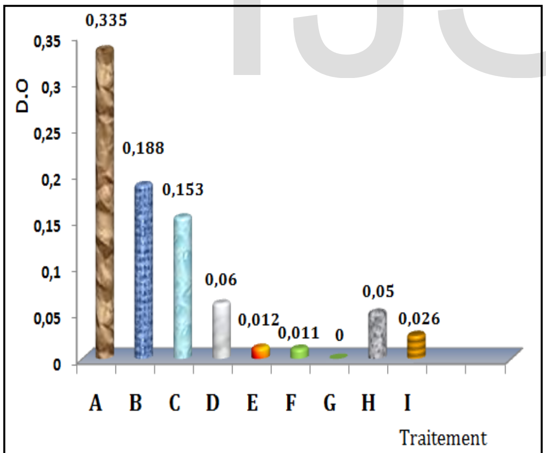

Figure 6: Change in absorbance of the P.C. to different treatments of OMWW

[**A**: P.C (OMWW 1/10),**B**: $Al_2(SO_4)_3$;**C**: $FeCl_3$; **D**: $Al_2(SO_4)_3$ and the addition of *Aspergilus Niger* ;**E**:$Fecl_3$ and the addition of *Aspergilus niger* ;**F**: $Al_2(SO4)_3$ and the addition of *Candida albicans* ;**G**:$Fecl_3$ and the addition of *Candida albicans* ;**H**: $Al_2(SO4)_3$ and the addition of *Candida tropicalis* ;**I**:$FeCl_3$ and the addition of *Candida tropicalis*]

3.5 Antibacterial activity

The disk diffusion method allowed us to highlight the antibacterial activity of OMWW on six bacteria (*E. coli, P. aeruginosa, Salmonella, Staphylococcus aureus, B.cereus, b. subtilis*). The results of this activity manifests itself differently on these bacteria tested by the development of different diameters of the zones of inhibition (between 9 and 16 mm), (table 6). We also found that this antibacterial effect is more important on bacteria Gram-positive (Halo of 11 to 16 mm) (Figure 7)

Table 6: Diameters (mm) of the OMWW inhibition zones

Bacteria	Raw OMWW	OMWW Diluted 1/4	OMWW diluted 1/2
B. cereus	17	15	12
S.aureus	16	15	14
B. subtilis	13	11	10
P.aeruginosa	12	10	10
Salmonella	10	9	9
E.coli	12	10	11

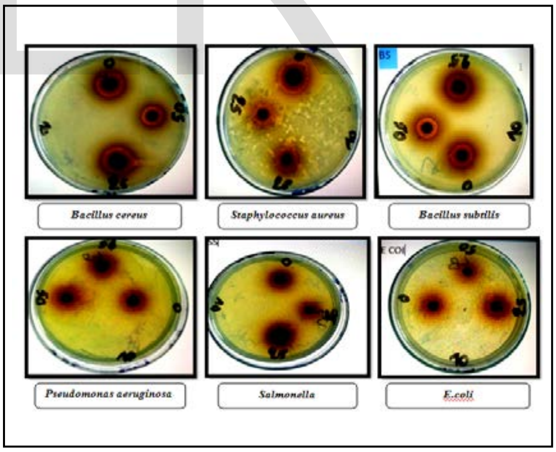

Figure 7: Antibacterial activity of OMWW tested bacteria

3.5.1 Antibacterial activity of P.C. extracts of OMWW

The P.C of OMWW would be the key factors of the antibacterial power. To confirm this deduction we tested the effect of the P.C on the multiplication of the bacteria tested (figure8).

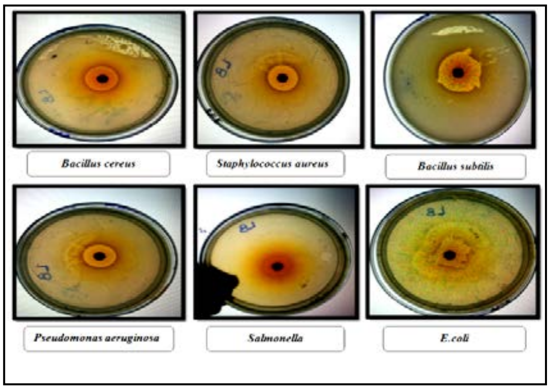

Figure 8: Antibacterial activity of P.C. extracts opposite the bacteria tested

These results the P.C confer to the vegetable water antibacterial power [21-26], because on the one hand these compounds are characterized by a very strong cross-linking and also a denaturation of proteins. The other hand because of their acid side chain [27]. Phenolic acids are much less polar. This property can facilitate the transport of these molecules through the cell membrane. They can be complexed with soluble extracellular proteins and also with bacterial walls as is the case of quinones. (More lipophilic) flavonoids can also disrupt the microbial membranes [28].

3.5.2 Antibacterial activity after treatment by coagulation-flocculation

The highlight of the antibacterial activity of OMWW after treatment by coagulation-flocculation is illustrated in figure 10. The results show a decrease in the aureoles of inhibitions in the OMWW untreated. This would be linked to a decrease in the P.C. by physico-chemical process.

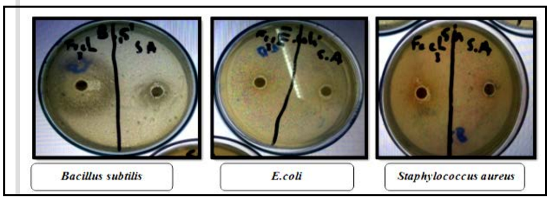

Figure 9: The antibacterial activity after treatment by coagulation-flocculation

3.5.3 Antibacterial activity after microbiological treatment

The results show a lack of halos of inhibitions for the bacteria tested. This indicates therefore that biological treatment can eliminate a lot of the still present P.C. in OMWW them treated by coagulation-flocculation.

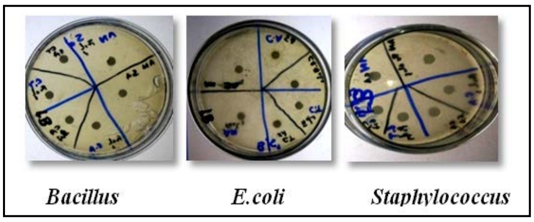

Figure10: Antibacterial activity after treatment by coagulation-flocculation

4 Conclusion

It emerges from this study that the OMWW reveals a microbial load rich in yeasts and mushrooms and also strong organic pollution shown particularly by the phenolic compounds. On the other hand The physico-chemical treatment process revealed a decrease of the turbidity, an increase of the muds volumes and an important discoloration of OMWW accompanied with a decrease of the rates of consisted phenolic. The second biological treatment showed a clear fall of the phenolic compounds. The physico-chemical and microbiological combined treatments of the OMWW could then answer the current tendencies which aim at the integration of diverse technologies to clean up OMWW with moderate cost.

Références :

[1]COI (Conseil Oléicole International).(2001). Le marché international de l'huile d'olive. Olivae 85, 22-25.

[2]ABHAT(2015) : centre national de documentation Royaume du Maroc Haut Commissariat au plan secrétariat d'état chargé de l'environnement « L'Industrie Oléicole au Maroc et son impact sur l'Environnement. Propositions d'actions de lutte contre la pollution générée par les Huileries d'Olives : Cas de la Province de Taounate » 2015.

[3] A. Cardinali, N .Cicco, V. Linsalata, F .Minervini, S. Pati, M. Pieralice, N. Tursi, V. Lattanzio (2010) Biological activity of high molecular weight phenolics from olive mill wastewater. J Agric Food Chem 58:8585‑8590.

[4]S.Sayadi, N.Allouche, M.Jaoua,F.Aloui (2000) Detrimental effects of high molecular mass polyphenols on olive mill wastewater biotreatment. Process Biochemistry. 35, 725-735.

[5]H.ElHajjouji,N.Fakharedine,G.A.Baddi,P.Winterton , J.R Bailly., J.C Revel. et M. Hafidi. (2007). Treatment of olive mill waste water by aerobic biodegradation: an analytical study using gel permeation chromatography, ultraviolet–visible and Fourier transform infrared spectroscopy. Bioresource Technology. 98: 3513-3520.

[6]I.Zenjari(2000). Etude éco-toxicologique des effluents liquides des huileries de la ville de Marrakech, impact sur les milieux récepteurs et détoxication. Thèse de Doctorat, Faculté des Sciences Semlalia, université Cadi Ayyad, Marrakech, Maroc. 149 p.

[7]G.Ouzounidou; GI .Zervakis, F. Gaitis (2010). Raw and microbiologically detoxified olive mill waste and their impact on plant growth. Terr Aquatic Environ Toxicol 4 (special Issue 1):21–38

[8]H.ElHajjouji, G.Ait Baddi, A.Yaacoubi, H.Hamdi, P.Winterton, J.C Revel.et M.Hafidi (2008). Optimisation of biodegradation conditions for the treatment of olive mill wastewater. Bioresource Technology. 99: 5505-5510.

[9]A.Achak, N.Ouazzni, A.Yaacoubi et L.Mandi, (2008). Caractérisation des margines issues d'une huilerie moderne et essai de leur traitement par coagulation-floculation par la chaux et le sulfate d'aluminium. Revue des Sciences de l'Eau. 21 : 53-57.

[10]V.Balice, C.Carrieri and O.Cera, (1990). Characteristic analytics of vegetation waters. The Italian Review of the Substances (Caratteristiche analitiche delle acque di vegetazione. La Rivista Italiana delle Sostanze Grasse LXVII 9-16.

[11] A.Dias, R.Bezerra and A.Nazaré Pereira, (2004). Activity and elution proile of laccase during biological decolorization and dephenolization of olive mill wastewater, Bio. Tech. 92:7–13.

[12]D.Mantzavinos and N.Kalogerakis, (2005). Treatment of olive mill efluents Part I. Organic matter degradation by chemical and biological processes an overview, Environ. Inter. 31:289–295.

[13]A.Jaouani, M.Vanthournhout and M.J.Penninckx, (2005). Olive oil mill wastewater puriication by combination of coagulation-locculation and biological treatments. Environ. Technol. 26 633-41.

[14]M.Achak, N.Ouazzani, A.Yaacoubi and L.Mandi, (2008). Caractérisation des margines issues d.une huilerie moderne et essai de leur traitement par coagulation-loculation par la chaux et le sulfate d ' aluminium. Sciences de l'eau. 2153 - 57.

[15]S.Khoui, F.Aloui and S.Sayadi, (2008). Extraction of antioxidants from olive mill wastewater and electro-coagulation of exhausted fraction to reduce its toxicity on anaerobic digestion. J. Hazard. Mater., 151: 531-539.

[16]P.Canepa, N.Marignetti, U.Rognoni and S.Calgari, (1988). Olive mills wastewater treatment by combined membrane processes. Water Res. 22: 1491-1494.

[17]E.S.Aktas, S.Imer and L..Ersoy, (2001). Characterization and lime treatment of olive mill wastewater, Water Res. 35:2336-2340.

[18]H.Dhaouadi and B.Marrot, (2008). Olive mill wastewater treatment in a membrane Bioreactor: Olive mill wastewater treatment in a membrane bioreactor: Process feasibility and performances.Chemical Engineering Journal. doi: 10.1016/ j. ce j. 04.017.

[19]E.DE Macro, S. Maria, P.Antonello and S.Raffaele (2007).Characterization and fractionation of phenolic compounds extracted from olive oil mill wastewaters. Food Chem , 104 : 858-867

[20]L.Della Grace, L.Previtera, F.Temessi, and A.Carrelli (2004). Lowmolecular weight components of olive oil mill waste-waters. Phytochem.Analys, 15 : 184-188.

[21] V. L Singleton and J.A Arossi (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Technol et Viticul ,16 : 144-153.

[22]S.KHOUFI, F.ALOUI, S.SAYADI (2000). Anaerobic digestion of olive mill waste water after Ca (OH)2 pretreatment and reuseadapted, Proc. Internat. Conf. on waste water treatment and reuse adapted to Mediterranean area (WATRAMA), 85-89.

[23]R.Capasso (1997). The chemistry, biotechnology and ecotoxicology of the polyphenols naturally occurring in vegetable wastes. Curr. Top. Phytochem., Res. Trends, 1, 145-156.

[24] J.M. Seng (1988).Chitine, chitosane et dérivés: De nouvelles perspectives pour l'industrie. Biofutur, 71, 40-44.

[25]Z.Ninnekar h., C.S. Vaidyanathan (1981). Catéchol 1,2-dioxygenase from Aspergillus niger: purification and properties. J. Indian Inst. Sci., 63, 131-136.

[26] A.Ranalli, (1991). Olivae, 37- 30.

[27]A.Esmail, H.Abed, M.Firdaous, N. Chahboun, Z.Mennane, E.Berny, et M.J Ouhssine, (2014). Mater. Environ. Sci.5- 121.

[28]A.Firas, F.Hassan, J. (2008). Zhejiang Univ. Sci. 9(2) , 154.