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An eco-compatible process for the depuration of wastewater from olive mill industry

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ABSTRACT

Olive mill wastewater (OMW) is the by-product of olive oil industrial production. It is characterized by a dark brownish color and a strong odor and is considered one of the most polluted agricultural wastes. In this paper we briefly describe an innovative procedure for the depuration of olive mill wastewater. With this procedure it is also possible to recover valuable substances such as phenolic compounds which have important commercial applications: they can be used in the prevention of cardiovascular disease and as antiviral, antioxidant and antitumor agents.

The proposed OMW treatment uses two different packed vegetable matrices which remove most of the pollutant substances by absorption. After filtration of OMW on the matrices the pollutant load of the waste is greatly reduced: the organic content (COD) is reduced more than 80% and the phenol compounds are completely removed.

Key words | biofiltration treatment, olive mill wastewater, phenol recovery, phenol removal

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INTRODUCTION

The extraction and use of olive oil has been an integral part of Mediterranean culture for over 6000 years (Civantos 1995; Tardàguila *et al.* 1996). Olive oil extraction involves a heavy consumption of water and produces large amounts of olive mill wastewater (OMW), the average volume ranging from 0.5 to 1.5 m^3 per ton of processed olives (Monteoliva-Sanchez *et al.* 1996; Paredes *et al.* 1996). In Italy, OMW is spread on cultivated fields under a strict law that strongly limits its use in agriculture (D.L. 574/96).

The fresh organic matter content in oil mill wastewater causes agricultural and environmental problems in olive oil-producing countries since its effects on soil status and fertility, insect proliferation and groundwater contamination are more harmful than beneficial (Cox *et al.* 1996; Spandre & Dellomonaco 1996). OMW contains a high amount of organic matter ($30-200\,\mathrm{kg\,COD\,m^{-3}}$), with a COD/BOD_5 ratio between 2.5 and 5, which is considered poorly degradable (Lopez 1992). The organic compounds in OMW (sugars, polyphenols, tannins, polyalcohols, pectins and lipids), in association with its high C/N ratio and low pH, compromise the biological degradation process of soils (Marques 2000) and can cause eutrophication when the wastewater is collected in basins with low exchange rates (closed gulfs, lakes, etc.).

The toxicity of OMW is also due to its high content of phenolic compounds in a wide range of molecular weights (MW), from low-MW substituted phenols to complex high-MW phenolic compounds (Montedoro *et al.* 1992). During olive oil production, large quantities of phenols are released along with the wastewater, according to their partition coefficient. Phenolics are derivatives of benzene (cyclic derivatives in the case of polyphenols) with one or more hydroxyl groups associated with their ring. The dark color of the water is caused by polyphenols (Pp) (Hamdi & Garcia 1993) and depends on the type of olives processed, their ripening stage, the climatic conditions and the technology used. However, despite their toxicity, polyphenols are used in the food, cosmetic and

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pharmaceutical industries on account of their high antioxidant activity.

The use of synthetic antioxidants in food processes can have negative health effects. Therefore, it is necessary to find natural substitutes that can inhibit the usual oxidization processes involved in the degradation of substances.

Treatment of OMW to recover valuable compounds like polyphenols could employ the aquatic fern *Azolla* as a biofilter. Dried *Azolla* biomass has already been used in the biosorption of a wide range of heavy metals from aqueous media (Sela & Tel-Or 1988; Cohen-Shoel *et al.* 2002). Pectin is an important polysaccharide constituent of *Azolla* cell walls, made up of fragments of polygalacturonic acid chains that interact with Ca^{2+} and Mg^{2+} ions to form a three-dimensional polymer (Schols *et al.* 1989; Jauneau *et al.* 1997; Kamnev *et al.* 1998).

As OMW consists of the diluted juice of crushed olives, it can be safely assumed that it is completely biodegradable. Yet even if all the constituents of OMW are biodegradable by definition, some of them, e.g. polyphenols and lipids, are decomposed at reaction rates much lower than others, e.g. sugars and short-chain volatile acids.

In recent years several methods have been proposed for OMW bioremediation, such as physical, physico-chemical or microbiological treatment. The physical and physico-chemical methods include thermal processes (evaporation and incineration), flocculation/clarification, ultrafiltration and reverse osmosis. Some of these systems have been patented (Knobloch *et al.* 2002; Pizzichini & Russo 2007).

The biological processes can be subdivided into anaerobic and aerobic ones. Nevertheless simple chemical or biological treatments cannot completely reduce OMW pollution and up to 85% of the organic substances, which could be recycled, are destroyed (Laconi *et al.* 2007).

The aim of this study was to test a new treatment for removing the pollution load in OMW by filtration on *Azolla* and granular activated carbon (GAC) to reduce the phenol, organic and inorganic matter content. This paper reports the results of laboratory experiments.

The filtration system is shown in Figure 1.

This method uses two different packed vegetable matrices (*Azolla* and GAC) which remove most of the pollutants by adsorption. Moreover, the phenolic compounds are almost completely recovered and the filtered water can be treated with calcium hypochlorite (ipoCa) to bring the COD to discharge values. The pretreated water can also be recovered for both hydrogen and biodegradable plastic production.

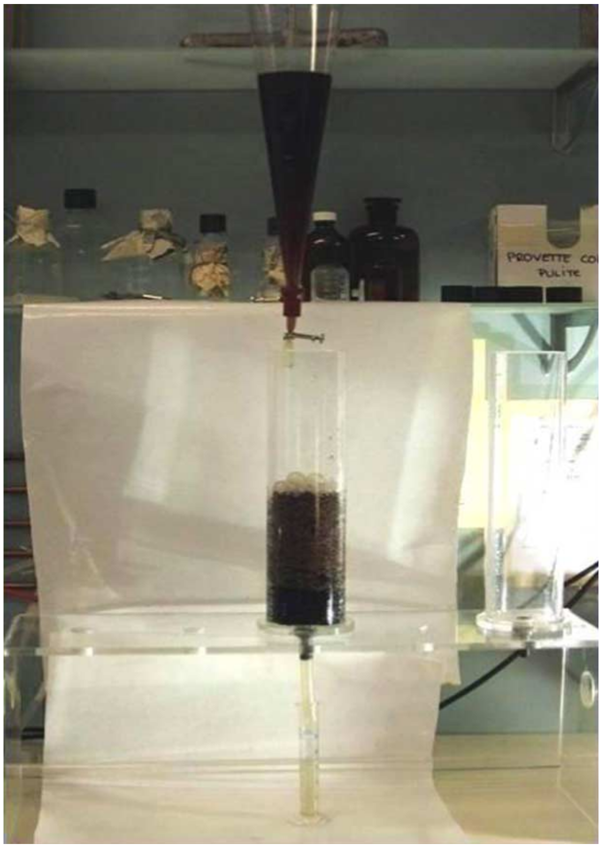

Figure 1 | Biofiltration system.

METHODS

Materials

Fresh olive mill wastewater samples were supplied by a continuous olive processing plant located in Bibbona in the province of Livorno (Italy). The samples, obtained from olives collected in January 2006 and immediately processed, were stored at −18°C. Citric acid, ethanol and all other chemicals were purchased from Sigma-Aldrich (St. Louis MO, USA). The multi-element and single element standards were supplied by CPI International (Santa Rosa CA, USA). The GAC (AFC-LS) was provided by Carboplant s.r.l. (Vigevano, PV, Italy).

Azolla cultivation

The strain of *Azolla caroliniana* derives from the Botanical Institute of Naples, Italy. Since the 1980s, it has been preserved by the Institute of Ecosystem Study (ISE), Florence section, of the Italian National Research Council (CNR). The *Azolla* biomass was cultivated in $2\,\text{m}^2$ vertical tanks containing a 10 cm layer of medium (reported by Ena *et al.* 2007), and then harvested and dried in the sun. This vertical apparatus permits to increase the cultivated surface from $6\,\text{m}^2$ to $14\,\text{m}^2$.

OMW pretreatment

Effluent (vegetation water) from a continuous olive oil extraction process was centrifuged. Table 1 shows the main characteristics of the OMW after centrifugation.

Each filtration experiment (in triplicate) was carried out twice and each value represents the mean of the six experiments. The pretreatment method uses two different packed vegetable matrices (*Azolla* and GAC) which remove most of the pollutant substances by absorption. The standard error was never higher than 5%. The results of this study demonstrate the feasibility of biofiltration on the vegetable matrices of wastewaters with an organic load of about 50–60,000 ppm.

Table 1 | Composition of the olive mill wastewater used in the present study

Property	Unit	Value
pH	pH	3.6
COD	mg/l O_2	52,500 ± 2,700
BOD_5	mg/l O_2	16,250 ± 800
Polyphenols	mg/Kg	4,005.0 ± 185
Chloride	mg/l	593.4 ± 27.2
Nitrate	mg/l	3.4 ± 0.18
Sulphate	mg/l	72.5 ± 3.5
Orthophosphate	mg/l	926.3 ± 46.2
Total P	mg/l	428.6 ± 18.6
Total N	mg/l	785.9 ± 35.8
K	mg/l	16.8 ± 0.77
Ca	mg/l	648.3 ± 32.0
Mg	mg/l	180.3 ± 9.1
Al	mg/l	1,257 ± 50.1
Cd	mg/l	<0.5
Cr	mg/l	33.2 ± 1.2
Fe	mg/l	6,535 ± 280
Hg	mg/l	<0.5
Ni	mg/l	925.3 ± 36.0
Pb	mg/l	7.8 ± 0.2
Cu	mg/l	335.2 ± 11.2
Zn	mg/l	4,588 ± 181

Main properties of the filtration supports

The adsorption properties of GAC and *Azolla* for the organic (COD) and phenolic compounds of OMW were determined in duplicate by placing 10 g of each support (previously sterilized in an autoclave) in anaerobic 100 ml-sterile bottles containing 20 ml of a filter-sterilized defined dilution (1:1, 1:2, 1:3, 1:5 or 1:8, in distilled water) of OMW. All bottles were brought to equilibrium conditions by shaking on a rotary shaker at 25°C and 150 rpm for 2 days. The adsorption data were calculated by measuring the residual concentrations of COD and Pp in the supernatants after filtration. Freundlich sorption kinetics for the organic matter and phenolic compounds in the immobilization carriers was then studied according to Colella *et al.* (1998).

Analytical methods

The analysis of BOD_5, total nitrogen, chloride, nitrate, sulfate, orthophosphate were carried out according to standard methods (Eaton & Greenberg 2005).

COD

The COD concentration in OMW was determined according to Ena *et al.* (2007).

Polyphenols

Polyphenols (with respect to gallic acid) were determined spectrophotometrically according to the Folin-Ciocalteau (Ena *et al.* 2007) using a Beckman DU 640 as spectrophotometer.

Total phosphorus

Total organic phosphorus was determined in unfiltered samples by acid digestion with potassium persulfate followed by colorimetric inorganic phosphate analysis with a UNICAM UV2 double ray spectrophotometer (Carmouze 1994).

K, Na, Mg

These ions were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) with an AGILENT 7500ce collision cell ICP-MS.

Heavy metals (Al, Cd, Cr, Fe, Ni, Pb, Cu, Zn)

The heavy metals were determined by atomic absorption with a UNICAM 939 graphite oven (confirmation by AGILENT 7500ce ICP-MS).

Hg

This metal was determined by atomic absorption spectroscopy at hydride development (PERKIN ELMER K16; confirmation by AGILENT 7500ce ICP-MS).

RESULTS AND DISCUSSION

Data on the growth of the aquatic fern (*Azolla-Anabaena azollae* symbiosis) outdoors under the climatic conditions of Florence are reported in Table 2.

The highest yield (14.2 g dry weight (d.w.) $m^{-2} d^{-1}$) was obtained in July; the average productivity was 10.3 g (d.w.) $m^{-2} d^{-1}$. The average planting density was 50 g (dry weight) m^{-2}.

A previous study had shown the ability of this aquatic fern (fresh biomass) to reduce phenol and organic matter in OMW (Ena *et al.* 2007). Other authors have used dried *Azolla* biomass in the biosorption of a wide range of heavy metals from aqueous media (Cohen-Shoel *et al.* 2002).

Table 2 | Yield and growth rate of *Azolla filiculoides* achieved outdoors in 2 m^2 vertical tanks

Months	Productivity (g (d.w.) $m^{-2} d^{-1}$)	Growth rate d^{-1}
July	14.2 ± 0.68	0.258 ± 0.009
August	10.5 ± 0.53	0.183 ± 0.009
September	7.2 ± 0.25	0.144 ± 0.005

Physico-chemical analyses (Table 1) of OMW showed that it was dark acidic waste with high levels of organic matter and polyphenols.

According to Ranalli (1992), phenolic compounds are the pigments responsible for the dark color of OMW. Moreover, OMW toxicity is mainly due to low molecular weight phenols (Della Greca *et al.* 2001); the toxicity of these compounds is caused by autoxidation processes (Nakai *et al.* 2001). This vegetation water also had high contents of chloride, orthophosphate, total P, total N, Ca and the heavy metals Ni and Fe.

As a first step, the two types of dried packed vegetable matrices were used separately: *Azolla* alone and GAC alone at two concentrations (50 and 100 g l^{-1}; 100 and 200 g l^{-1} respectively). The COD and Pp removal results (Figure 2) show that the lower concentrations were most efficient (220 and 25 mg g^{-1} respectively), while the values were somewhat lower (162 and 20.5 mg g^{-1} respectively) at 100 and 200 g l^{-1}. However, the higher concentrations removed greater amounts in absolute.

Considering the great chemical heterogeneity of OMW, we studied the adsorption properties of GAC and *Azolla* in terms of removal "broad parameters" instead of determining adsorption isotherms for single compounds in the OMW. The Freundlich adsorption isotherm, $q_e = k.Ce^{1/n}$ (q_e = adsorbed COD or phenols per unit mass of solid material, mg g^{-1}, and Ce = COD or phenolic compound concentration at equilibrium, mg l^{-1}), was used to represent the COD and phenolic compound adsorption on both supports. Thus, the characteristic Freundlich parameters (k and $1/n$) are best regarded here as descriptive and they

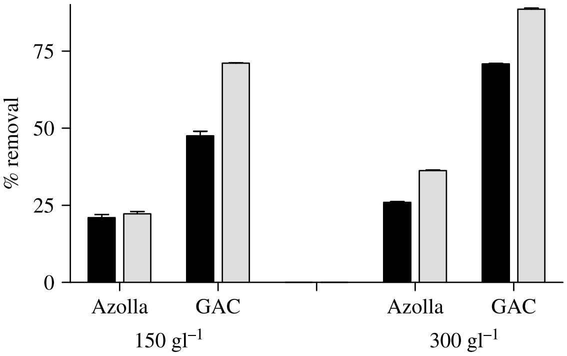

Figure 2 | Abatement percentages of COD (black bars) and Pp (grey bars) in alone (at two different concentrations) vegetable matrices.

Table 3 | Characteristic Freundlich parameters

	k(mg/g)/[(mg/l)$^{1/n}$]	1/n	Correlation coefficient
COD *Azolla*	2.60E-03	1.0482	0.9963
Pp *Azolla*	5.42E-03	1.1102	0.8941
COD GAC	2.63	0.5186	0.9997
Pp GAC	8.08	0.4188	0.9791

were optimized by non-linear least-square regression of experimental adsorption data (Table 3).

The experimental adsorption data and calculated Freundlich isotherms for COD and phenol adsorption are compared in Figure 3a and b for *Azolla* and Figure 3c and d for GAC.

As expected, the total organic matter (measured as COD) and phenolic fraction of OMW were both much more efficiently adsorbed by GAC (70.8% and 88.5% respectively) than by *Azolla* (25.95% and 36.25% respectively).

The different vegetable matrices were then tested together by packing one on top of the other in a column (concentrations: 150 g l^{-1}, 300 g l^{-1}, 360 g l^{-1}). The results indicate that the concentrations of 300 g l^{-1} and 360 g l^{-1} provided greater removal of phenols and organic matter: COD removal increased to 80.5% and 84.5% respectively and phenol absorption to more than 99% in both conditions (Figure 4).

A higher concentration of vegetable matrices used in the biofilter (360 g l^{-1}) do not provide a significant removal of pollutant substances (Pp and COD) if compared to that obtained with the 300 g l^{-1} concentration, considering the increase of the matrices utilized. Therefore we have used the 300 g l^{-1} concentration. The progress of the treatment process is shown in Figure 5: 40% of the organic load of COD was removed by centrifugation and over 80% by biofiltration; 12% of Pp was removed by centrifugation and over 99% by biofiltration.

The percentage removal data for the alkaline metals (K, Ca and Mg) after biofiltration demonstrate a higher affinity of matrices for K and Mg (more than 70%) while the removal of Ca was very poor (47%). When several components are present, interference and competition for adsorption sites probably occur, leading to a lower saturation concentration of the adsorbed component. Moreover, other substances such as total nitrogen and

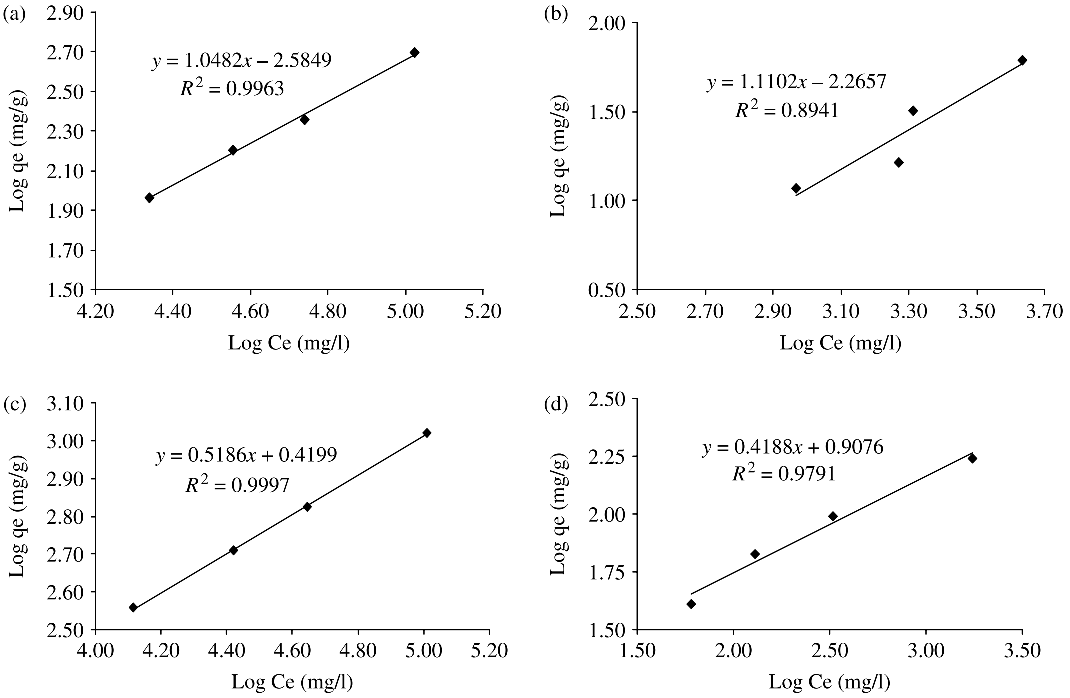

Figure 3 | COD (a, c) and phenolic compounds (b, d) Freundlich isotherms on *Azolla* (a, b) and GAC (c, d).

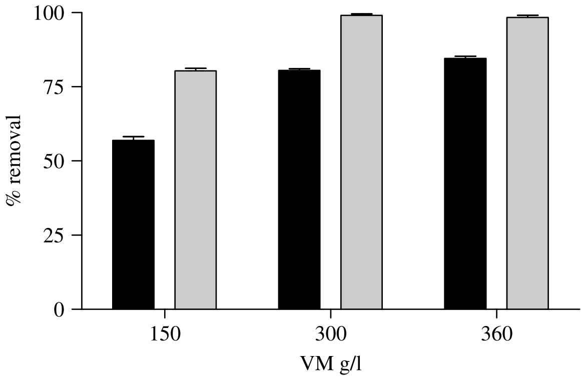

Figure 4 | Removal percentages of COD (black bars) and Pp (grey bars) at different concentrations of together packed vegetable matrices.

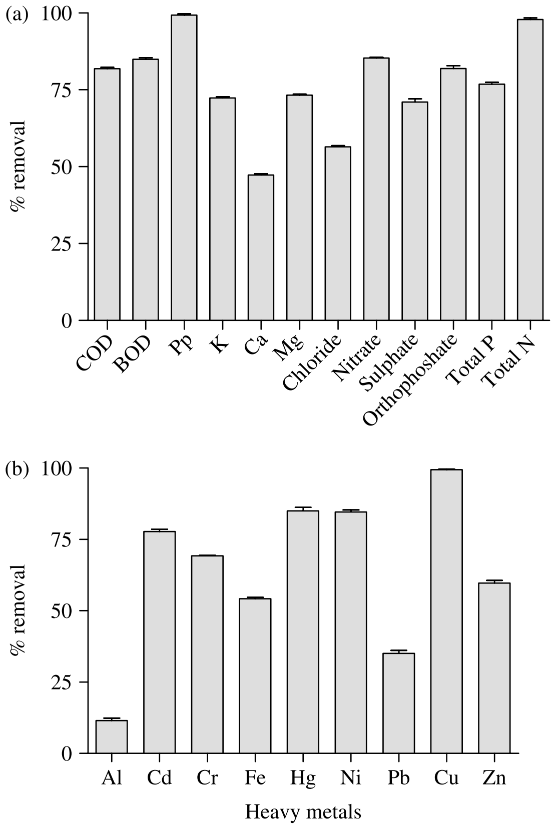

Figure 6 | Removal percentages of some chemical parameters (a) and heavy metals (b).

copper were almost totally absorbed by the matrices while different metallic compounds (Al and Pb excepted) were variably reduced by 50–85% (Figure 6a and b).

The heavy metals with higher ionic charge probably cannot be completely adsorbed by the vegetable matrices according to the aforesaid phenomena.

For complete removal of the COD, the residual waste after biofiltration was treated by oxidation with different concentrations of calcium hypochlorite (1,800–15,760 ppm). As shown in Figure 7, the highest COD removal (366 $\mathrm{mg\,g^{-1}}$ of ipoCa) was obtained in the experiment with the highest initial concentrations of both COD and ipoCa; however this treatment was not effective because the organic load remained and there was also a large amount of chlorides.

The best treatment was with the lower initial contents of COD and ipoCa: the COD concentration was reduced by 92%, giving a final concentration of 150 $\mathrm{mg\,l^{-1}}$ which is compatible with Italian law (160 $\mathrm{mg\,l^{-1}}$ COD in surface waters). Figure 8 illustrates the complete treatment.

The results obtained with this treatment are comparable to those using membrane technologies (ultrafiltration, microfiltration, nanofiltration and reverse osmosis) (Russo 2007), although our method is much more economical.

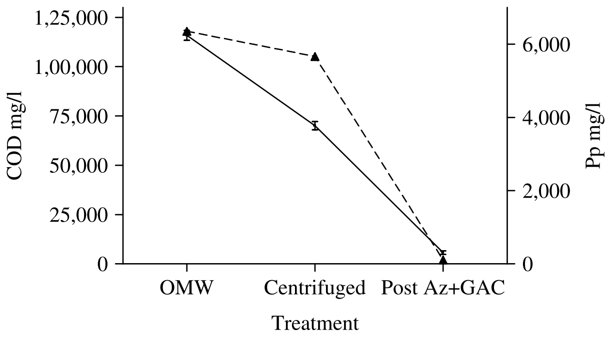

Figure 5 | Removal of COD (continuous line) and Pp (discontinuous line) in the invented treatment.

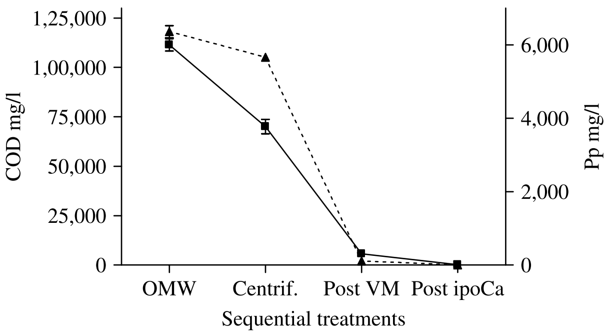

Figure 7 | Complete treatment: the abatement of COD (continuous line) and Pp (discontinuous line).

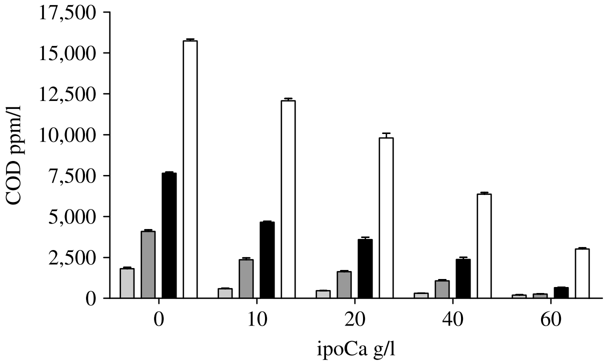

Figure 8 | Removal of COD in treated OMW with different initial COD concentrations (1,800 ppm light grey bars, 4,080 ppm grey bars, 7,680 ppm black bars, 15,760 ppm white bars) and different Ca ipoclorite concentrations.

The pretreated wastewater (after biofiltration) can be used for various applications.

(i) Polyphenol recovery by vegetable matrix. Phenols are very interesting compounds as they can be used in the prevention of cardiovascular disease and as antiviral, antioxidant and antitumor agents. They can also be used for food, cosmetic and pharmaceutical applications. In particular, OH-tyrosol shows strong antioxidant, antinflammatory and antiviral activity (Ohno *et al.* 2002), higher than that of many other compounds (Manna *et al.* 1999; D'Angelo *et al.* 2005). The total desorption of polyphenols from the exhausted matrices is about 50–60%.

(ii) The pretreated wastewater can be used as a substrate for the growth of a purple bacteria (*Rhodopseudomonas palustris*) able to metabolize residual organic compounds (mainly short-chain organic acids) for biohydrogen production. Under the same conditions, H_2 production experiments (conducted in free nutrient OMW) were carried out in both batch and discontinuous mode: removing and adding a fixed amount of wastewater. The results showed that in batch conditions the biogas production was ca. 600 ml per liter of culture, while in experiments carried out in discontinuous mode for more than 300 hours the yield was ca. 1,200 ml per litre of culture (data not shown). These results are very good compared with those reported by Eroglù *et al.* (2004) because in that paper optimal condition OMW was used at 2% dilution.

(iii) When growing in unbalanced conditions, *R. palustris* can produce biopolymers (polyhydroxyalkanoates) with potential applications in medicine and surgery as biodegradable plastic material. In these microorganisms the polyester (PHB) is used as an energy reserve compound.

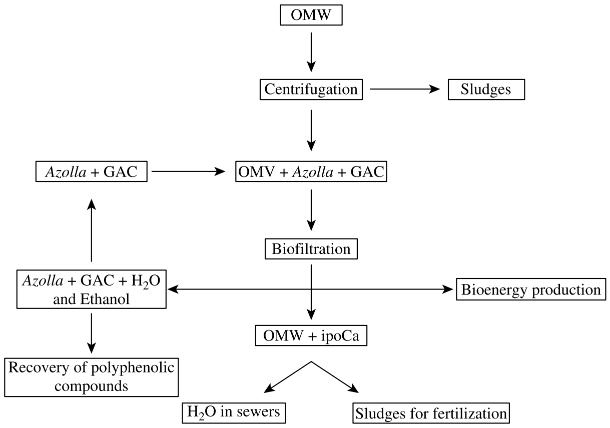

Figure 9 | Complete treatment plan of the olive oil mill wastewater.

In the end the treatment proposed according to the present research not only provides for abating the pollution impact of olive mill wastewater, so that it can be directly disposed in the environment with full compliance of current legislation, but also uses this OMW as a raw material obtaining the following commercially useful products: organic fertilizers, antioxidant compounds and water that can be used for all oil press customs. Figure 9 sets out schematically the complete treatment suggested in this paper.

CONCLUSIONS

This paper is the first description of a complete biodepuration treatment of olive mill wastewater carried out with a biofiltration system using vegetable matrices. The pretreated water can be utilized for various applications. For example, the OMW pretreated with the described method is a promising substrate for the biological production of valuable by-products (biohydrogen, polyhydroxyalkanoates and pigments). Consequently, the water obtained after the suggested applications can be utilized for all oil press uses.

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