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Extraction of antioxidants from olive mill wastewater and electro-coagulation of exhausted fraction to reduce its toxicity on anaerobic digestion

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Abstract

Liquid–liquid extraction was used in order to recover phenolic compounds from centrifuged olive mill wastewater (OMW), a polluting by-product of olive oil production process, and to reduce their toxicity for a subsequent aerobic or anaerobic digestion. Phenolic compounds were identified in untreated and treated OMW by gas chromatography coupled to mass spectrometry (GC–MS). The experimental results of ethyl acetate extraction showed that the monomers recovery efficiency was over 90%. This pre-treatment resulted in the removal of the major LMM phenolic compounds and a small part of HMM polyphenols. The aerobic treatment of the exhausted OMW fraction removed 78.7% of the soluble COD. In the case of anaerobic digestion at OLR ranged from 1 to 3.5 g COD l^{-1} day^{-1}, methanisation process exhibited high methane yield as 0.31 CH$_4$ produced per g COD introduced and high COD removal (80%). However, a disruption of the process was observed when the OLR was increased to 4.5 g COD l^{-1} day^{-1}. A pre-treatment by electro-coagulation resulted in decreasing the toxicity and enhancing the performance of methanisation operated at higher OLR from 4 to 7.5 g COD l^{-1} day^{-1}.
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Keywords: Olive mill wastewater; Polyphenols; Extraction; Antioxidant; Anaerobic digestion; Electro-coagulation

1. Introduction

A major environmental concern in the Mediterranean countries is the disposal and/or treatment of the large quantities of olive mill wastewater (OMW) produced during olive oil processing. The high-polluting power of OMW is generally associated with the high biochemical oxygen demand (BOD), chemical oxygen demand (COD), total solids, organic carbon and the slightly acidic character of OMW [1,2]. Regarding the organic load of OMW, phenolics and related compounds were reported to be present in very high concentrations. This wastewater constitutes a serious problem with severe negative impact on soil and water quality, and thus on agriculture, environment and health [3]. Therefore, it is not surprising that research efforts have

been directed towards the development of efficient treatment technologies including several physical, chemical and biological processes as well as various combinations of them; these technologies have recently been reviewed by Mantzavinos and Kalogerakis [4].

On the other hand, OMW contain phenolic compounds which possess ideal structural chemistry for free radical-scavenging and metal-chelating properties, and have been shown to be more effective antioxidants *in vitro* than vitamins E and C on a molar basis [5]. The interest in natural antioxidants is increasing due to the evidence for the involvement of oxygen-derived free radicals in several pathological processes [6]. Epidemiological studies have suggested a connection between the consumption of polyphenol-rich food and the prevention of diseases associated with oxidative stress, such as cancers, cardiovascular diseases, inflammations and others. Overall these notes, OMW could represent an interesting and alternative source of biologically active polyphenols.

Environmental issues for the claimed soil contamination and economic ramifications raised the need for detoxification of OMW, as well as recovery and exploitation of by-products

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at all stages of olive oil industry. In light of the aforementioned environmental issues, Mediterranean countries which mainly produce OMW are encouraged to develop new technologies regarding the reduction of their polluting load. This could be combined with the possible exploitation of polyphenols, the main bioactive components of OMW, endowed with antioxidant, anti-inflammatory and other biological activities [7].

Several researches have evaluated the feasibility and economic processes for recovering olive phenols from OMW or solid wastes [8–11]. Principal systems proposed to recover the phenols from OMW are: extraction with solvents; resin chromatography; selective concentration by ultra-filtration and reverse osmosis; solid–liquid or liquid–liquid extraction, supercritical fluid extraction [12,13]. In previous investigations, it was reported that among all procedures that are employed for natural antioxidants recovery, liquid–liquid solvent extraction represents a simple and convenient alternative, and it is widely used in pilot-scale production and in ultimate commercial recovery [14]. This technique resulted in a polyphenol rich extract and an exhausted fraction (residue) which is a complex mixture of water, sugars, nitrogenous substances, organic acids, pectins, mucilages, residual polyphenols and tannins, lipids and inorganic substances. Physico-chemical or biological treatment of this exhausted fraction is necessary before discharging into natural stream.

In this study we investigated the liquid–liquid extraction of OMW for recovering the amount of natural antioxidants present. The phenolic extract was analysed by GC–MS. The second step of this study is directed to study the biological treatment (aerobic and anaerobic digestion) of the exhausted fraction. Electro-coagulation treatment was tested in order to improve the methanisation of the effluent.

2. Materials and methods

2.1. OMW characterization

OMW was obtained from a discontinuous olive oil processing plant located in Sfax (southern Tunisia). Fresh OMW was withdrawn and stored at $-20\,°C$. OMW was centrifuged (20 min at 4000 rpm) to remove solids, prior to experiments. The main characteristics of this OMW sample were illustrated in Table 1.

2.2. Extraction of phenolic compounds from OMW

Continuous counter-current extractions were conducted at ambient temperature in a polyethylene mixer-settler unit of ROBATEL design [15]. The total feed flow rate ranged from 2 to $5\,l\,h^{-1}$. The exhausted fraction (OMW without LMM phenolic compounds) was left under gentle agitation in order to evaporate the traces of solvent before anaerobic digestion. The extract was analysed by GC–MS.

2.3. Aerobic treatment

The biological sludge used for the aerobic digestion of the exhausted OMW fraction was collected from the municipal wastewater treatment plant located in Sfax (Tunisia) and acclimatized for 2 months on diluted OMW. Total solids (TS) content of the sludge was adjusted to $18.0\,g\,l^{-1}$ for all experiments.

Aerobic biodegradation experiments were performed in 500 ml flask containing 200 ml of diluted OMW sample (25%) as sole carbon source (acclimatized sludge is not able to grow on raw exhausted OMW). The pH of the medium was adjusted to 7.0. The flasks were incubated in an orbital shaker at $150\,rpm\,min^{-1}$ at ambient temperature. The tests were conducted in duplicate. The evolution of pH and soluble COD was analyzed every day. Results of all analysis represent the mean values of replicate trial degradations.

2.4. Anaerobic digestion

Three anaerobic filters (AF) were used in this study (Fig. 1). These reactors were made of glass columns having working volumes of 3 l. The inner tubes were enclosed in a jacket through which hot water circulated to maintain the temperature of the filter at $37\,°C$. These anaerobic filters were packed with polyurethane foam cubes $2\,cm \times 2\,cm \times 1\,cm$ (Filtren T45, from Recticel, Wetteren, Belgium) as support and inoculated with an 8-year-old digester operated with pre-treated OMW. The influent was fed in six times into the reactor using a pump connected to a programmer. For monitoring the volatile fatty acids (VFA) inside the reactor, three sampling points were made in the AF. Level (A) was at the bottom of the reactor. Level (B) corresponded to the middle and level (C) was at the top of the reactor (Fig. 1). Total VFA was measured by HPLC using the method described by Mechichi and Sayadi [16].

Table 1

Composition of crude OMW, the exhausted fraction (S1), exhausted fraction after electro-coagulation–sedimentation treatment (S2) and the anaerobic effluent of electro-coagulated exhausted OMW fraction (S3)

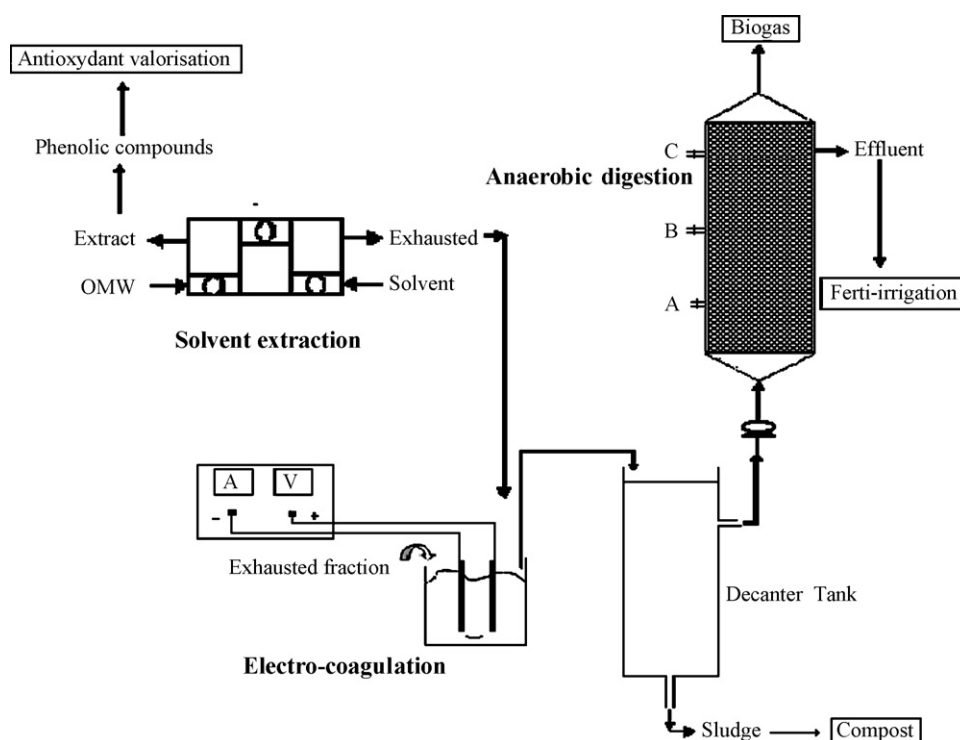| Parameters	Crude OMW	S1	S2	S3
pH	5.23 ± 0.1	2.30 ± 0.15	7.80 ± 0.1	8.1 ± 0.23
Soluble COD $(g\,l^{-1})$	119.5 ± 2.5	98.0 ± 3.27	38.5 ± 1.2	4.3 ± 0.5
COD/BOD$_5$	5.8	3.4	2.3	2.8
Ortho-diphenols $(mg\,l^{-1})$	6640.0 ± 15	3700.0 ± 13	820.0 ± 5	92.0 ± 1.5
Total polyphenols $(g\,l^{-1})$	11.0 ± 1.5	4.2 ± 0.5	1.1 ± 0.2	ND
Residual oils $(g\,l^{-1})$	12.0 ± 1.7	11.5 ± 1.5	0.97 ± 0.14	0.0
TSS $(g\,l^{-1})$	29.7 ± 2.2	0.0	2.6 ± 0.25	1.5 ± 0.15
Turbidity (NTU)	10567 ± 60	254 ± 17	460 ± 20	172 ± 12

Fig. 1. Experimental procedure of OMW treatment by solvent extraction, electro-coagulation and anaerobic digestion.

The biogas production of the anaerobic filters was measured by liquid displacement. Gas samples were taken with a syringe from the tank of biogas. CH_4, CO_2 and N_2 were measured using a gas chromatograph GC11 [17].

2.5. Electro-coagulation treatment

The electro-coagulation reactor (5 l glass) was formed by one pair of anodic and cathodic electrodes (cast iron plates) which were positioned approximately 4.5 cm apart from each other and were dipped in the effluent. The total effective surface area of electrodes was 0.3 dm^2. The current input was supplied by a Convergy power supply. In each run, 3 l of OMW fraction was placed in the electrolytic cell. This electrolysis process lasted 4 h at 1.7 A dm^{-2}. After electro-coagulation, treated effluents were placed in a decanter tank (Fig. 1) to eliminate sludge formed during electrolysis.

2.6. Analytical methods

BOD$_5$ was determined by the manometric method with a respirometer (BSB-Controller Model 620 T (WTW)) and the chemical oxygen demand (COD) was estimated using the method described by Knechtel [18]. Ortho-diphenols concentration was determined according to Folin Ciocalteau method [19]. Total polyphenols were quantified by ethyl acetate method as described by Sayadi et al. [20]. The standard method of Soxhlet solid/liquid (organic solids of OMW/hexane) was utilised for the dosage of lipids.

Gel Filtration method was used to analyse molecular-mass distribution of the OMW polyphenols. Samples (2 ml) were fil-

tered and placed on a Bio-gel P6 column previously equilibrated with a borate tampon (pH 9). The flow rate was adjusted to 3.3 ml min^{-1}, and 3 ml fractions collected. These fractions were measured spectrophotometrically at 280 nm. The column was calibrated with bleu dextran (2000 kDa), RNase (30 kDa) and p-hydroxyphenylacetic acid (152 Da).

GC–MS analysis was performed with a HP model 5975B inert MSD, equipped with a capillary DB-5MS column (30 m length, 0.25 mm internal diameter, 0.25 mm film thickness (Agilent Technology, J & W Scientific Products, USA)). The carrier gas was He used at 1 ml min^{-1} flow rate. The oven temperature program was as follows: 1 min at 100 °C, ramped from 100 to 260 °C at 4 °C min^{-1} and 10 min at 260 °C. The chromatograph was equipped with a split/splitless injector used in the split mode. The split ratio was 100:1.

OMW samples (40 ml) were acidified at pH 2 by HCl (1N) and extracted with ethyl acetate (4/40 ml). The organic layer was collected and reduced to 10 ml by rotary evaporation (37 °C) and then silylated. For the silylation procedure, a mixture of pyridine (40 μl) and BSTFA (200 μl) were added and vortexed in screw cap glass tubes and consecutively placed in a water bath at 80 °C for 45 min. From the silylated mixture 1 μl was directly analyzed by GC–MS.

3. Results and discussion

3.1. Solvent extraction

Solvent extraction of valuable compounds, particularly antioxidants such as hydroxytyrosol was considered as an alternative for valorising this by-product at international level

[15,21]. Besides its intrinsic economic interests, recovery of LMM might be beneficial for the ensuing reduction in OMW phenolic content in view of further downstream treatments and/or applications. One of the priorities of this study was to integrate the solvent extraction as a stage of OMW pre-treatment and antioxidant recovery, prior to a conventional bio-treatment.

Before the extraction step, OMW was centrifuged to eliminate all suspended solids, such as colloids, pigments, and impurities. In this study, extraction of OMW was carried out in a polyethylene mixer-settler unit of ROBATEL using ethyl acetate as reported in previous studies [11,15]. This OMW extraction process resulted in an ethyl acetate extract containing the target compounds and an exhausted fraction representing the aqueous phase of OMW for which 90% of extractable mono-phenolic compounds were recovered. This fraction is destined for a biological post-treatment.

For the extraction process, the main cost is the consumption of the extracting solvent. Thus, a significant cost reduction is achieved because the solvent can be recycled. Ideally, when a mixture of two liquids is distilled, the substance with the lower boiling point vaporizes first and can be collected separately from the second substance. The HPLC results showed that the purity of recycled ethyl acetate was as high as 98% (data not shown). The distillation residue containing phenolic compounds could be further purified by chromatography techniques.

3.1.1. Analysis of OMW extract

Phenols recovered by ethyl acetate extraction were dried by rotovapor and dissolved in a fixed volume of ethyl acetate for GC–MS analysis. Concentration of total OMW polyphenols was $11\,g\,l^{-1}$. Concentration of phenolic compounds recovered from OMW was in the range of $6\,g\,l^{-1}$ (Table 1). The GC–MS chromatogram of the final extract, obtained from OMW using the continuous counter-current extractor is shown in Fig. 2a. The identification of the different peaks of the gas chromatogram was achieved from the mass spectra of their trimethylsilylated derivatives (Table 2), analyzed by the means of the GC–MS apparatus, and by comparison to reference compounds. The identified phenolic compounds were: tyrosol, hydroxytyrosol, homovanillic acid, cafeic acid, *para*-coumaric acid and ferulic acid. Other compounds such as fatty acids and reducing sugars were also detected (Fig. 2a). Hydroxytyrosol was the major compound and it represented 66.5%. It is known from literature that among these phenolic compounds only hydroxytyrosol and cafeic acid exhibited good antioxidant properties, but in all cases, tyrosol
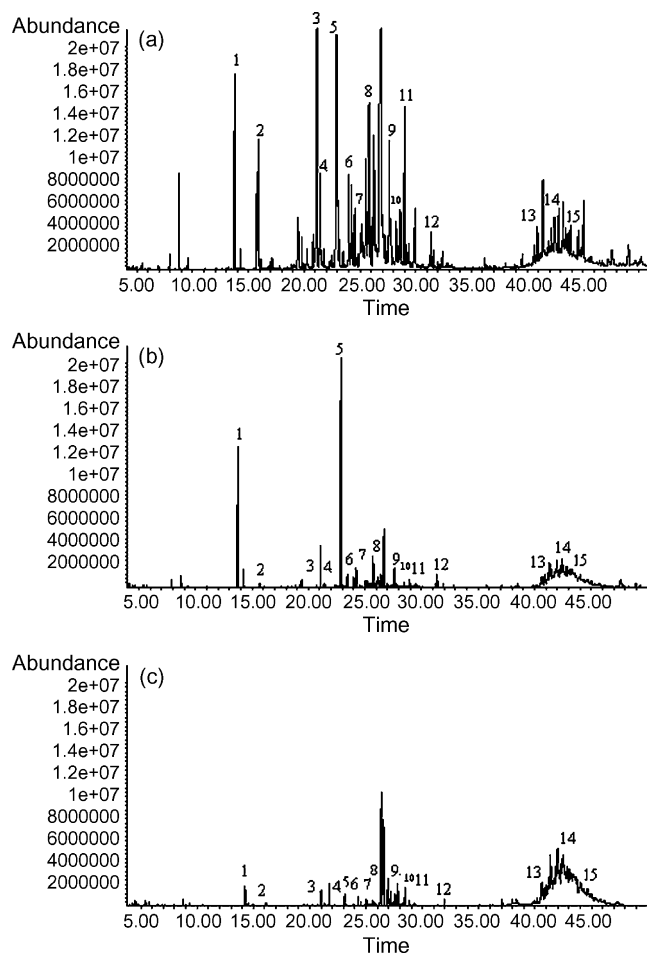

Fig. 2. Total ion gas chromatogram of ethylacetate-extractable products obtained from acidified OMW (a), exhausted fraction (b) and aerobic treated OMW (c). The MS-identified compounds with respect to their retention time are the following—13.75: malic acid (1); 15.72: tyrosol (2); 21.15: hydroxytyrosol (3); 22.45: protocatechuic acid (4); 22.98: citric acid (5); 24.21: 3,4-dihydroxymandelic acid (6); 25.19: *para*-coumaric acid (7); 26.22: homovanillic acid (8); 27.59: palmitic acid (9); 28.54: ferulic acid (10); 29.94: cafeic acid (11); 31.39: oleic acid (12); 40.91: arabinofuranose (13); 42.46: β-D-glucopyranoside (14); 43.88: α-D-glucopyranoside (15).

and ferulic acid are considered to be nutraceutically positive [22].

Therefore, the recovery of antioxidants from OMW is of great importance, not only because of their aforementioned significant properties, but also because it could exploit a large amount of OMW. Previous results showed that olive phenols recovered

Table 2
Abbreviated mass spectra of the main phenolics recovered from OMW by ethyl acetate extraction

| TMS derivatives of	Mass spectra
Tyrosol	282 (M^+); 179; 147; 126; 103; 73
Hydroxytyrosol	370 (M^+); 323; 267; 237; 193; 147; 103; 73
Protocatechuic acid	370 (M^+); 341; 311; 281; 223; 193; 165; 137; 105; 73
3,4-Dihydroxymandelic acid	443 (M^+); 355; 281; 251; 207; 177; 147; 117; 73
para-Coumaric acid	293 (M^+); 249; 219; 179; 139; 115; 73
Homovanillic acid	326 (M^+); 296; 267; 237; 209; 179; 149; 125; 104; 73
Ferulic acid	338 (M^+); 323; 308; 293; 279; 249; 219; 191; 175; 160; 146; 73
Cafeic acid	425 (M^+); 396; 354; 307; 278; 249; 219; 179; 147; 115; 73
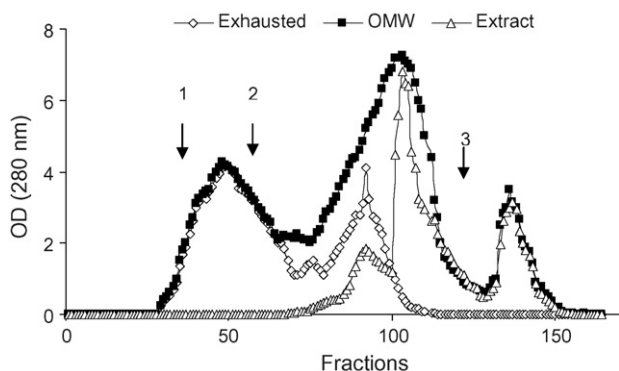
Fig. 3. Molecular-mass distribution of polyphenolics of the crude OMW, the exhausted fraction and the ethyl acetate extract by gel filtration on Bio-gel. 1: blue dextran (MM = 2000 kDa), 2: RNase (30 kDa), 3: *p*-hydroxyphenylacetic acid (152 = Da).

by ethyl acetate extraction from OMW are good antioxidants for lard [23]. Others studies showed that OMW polyphenols have an antioxidant effect on intestinal human epithelial cells [24] and a cytostatic action on some tumour cells [25]. Fki et al. [11] showed that OMW extract can be used as alternative natural antioxidants to stabilize edible oils, while at the same time appeasing a major concern of consumers over the use of synthetic antioxidants in food products.

3.1.2. Characterisation of the exhausted fraction

Gel filtration of OMW before and after ethyl acetate extraction showed that ethyl acetate was able to extract the major fraction of LMM phenolics and a small part of the oligomeric fraction (Fig. 3). Removal of mono-aromatics by ethyl acetate extraction was proved by GC–MS analysis (Fig. 2b). The decrease of these compounds led to an increase of BOD_5 of the exhausted fraction in comparison with the initial BOD_5 of raw OMW. Biodegradability is determined by measuring the ratio between COD and BOD_5, whose value must be in the range of 2–2.5. After ethyl acetate extraction, biodegradability decreased from 5.8 to 3.4 (Table 1). Yet, this ratio was still higher than the requirements. This confirms that a part of non-biodegradable phenolic compounds is still present in the exhausted fraction. Total polyphenols and ortho-diphenols concentrations in the exhausted fraction exhibited a reduction of more than 50% (Table 1).

The objective of ethyl acetate extraction pre-treatment of OMW was the recuperation of antioxidants and the decrease of OMW toxicity. The major part of the residual COD of the exhausted fraction was to be eliminated by the biological post-treatment.

3.2. Aerobic treatment

Bioremediation experiment has been carried out in shaking batch cultures, using an activated sludge previously acclimatized on OMW. The process was investigated under non-sterile operating conditions, representative of large-scale operation. OMW bioconversion was characterized in terms of soluble COD removal and pH evolution as a function of time.
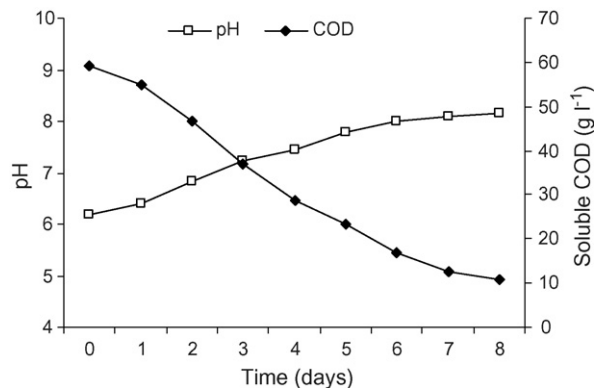

Fig. 4. Evolution of pH and soluble COD of OMW exhausted fraction during 7 days of aerobic digestion.

Fig. 4 illustrates the variation of pH and soluble COD during the batch aerobic degradation process of the diluted exhausted fraction (25%). It is noted that the residual solvent present in the exhausted fraction (7%) increased the value of COD. As can be seen in Fig. 4, the soluble COD removal efficiency of the exhausted fraction was 78.7% after 7 days of digestion time. The high organic matter removal efficiency may be attributed to the reduction in the toxic phenolic compound concentration, which decreased from 11.00 to 1.05 g l^{-1} after extraction and dilution. At the end of the aerobic treatment, the pH increased to a range of 8–8.5 after 7 days of aerobic treatment. Samples withdrawn prior to and after treatment were analyzed by means of GC–MS and representative chromatograms are shown in Fig. 2b and c. The comparison of these chromatograms pointed out that the aerobic digestion was able to degrade all the residual mono-phenolics (when exhausted fraction was diluted at 25%).

It has been reported that phenol concentrations in excess of 50 mg l^{-1} inhibit the biodegradation rate [26].

3.3. Electro-coagulation pre-treatment of the exhausted fraction

Electro-coagulation is one of the simple and efficient electro-chemical methods for the purification of several types of water and wastewaters. During electro-coagulation, when a potential difference is applied between a soluble anode, such as Fe or Al and the cathode, ferrous or aluminum and hydroxyl ions are generated, respectively, at the anode and the cathode. This process is followed by in situ oxidation to the ferric state and subsequent precipitation as ferric or aluminium hydroxide. In this study, treatment of the exhausted fraction by electro-coagulation using iron electrodes was investigated in order to improve the anaerobic digestion.

The electro-coagulation step was performed on OMW exhausted fraction. The effect of the electrolysis treatment on OMW treatment was investigated by the measurement of pH, COD, BOD_5, turbidity, TSS and phenol content. The characteristics of the exhausted fraction before and after electro-coagulation are illustrated in Table 1. It should be noted that the pH of effluent increased to 7.8 during electrolysis treatment. After electro-coagulation, the soluble COD of OMW

drops to approximately 60.7% of the initial value. This result points out the ability of the electrolysis process to remove a wide range of soluble compounds present in OMW. The COD/BOD$_5$ ratio was decreased from 3.4 to 2.3. It appears therefore that a significant proportion of the non-biodegradable matter present in OMW (mainly polyphenols) was removed by electro-coagulation. The concentration of ortho-diphenols, monitored by Folin–Ciocalteau method, was significantly reduced during the electro-coagulation process. Phenol removal efficiencies were about 73.8% for total polyphenols and 77.8% for ortho-diphenols (Table 1). Moreover, chromatography analysis confirmed the removal of most HMM phenolics (data not shown). The reduction in wastewater phenolic content could be attributed to the polymerisation occurrence of HMM polyphenolic compounds and to the physical and/or chemical adsorption of phenols to solid particles in the remaining sludge as demonstrated in a previous study [27]. Furthermore, as can be seen in Table 1, the concentration of lipids was decreased by 92%. In this study it is suggested that a simple sedimentation process (Fig. 1) would be able to improve the OMW quality for the subsequent anaerobic post-treatment mainly in terms of TSS and turbidity [27]. This result confirms the hypothesis that the electro-coagulation would have a strong ability to eliminate the

residual HMM polyphenols from the exhausted fraction. The pH, COD, coloration, polyphenols and lipids removal were consistently high. Indeed, the effluent quality of the pre-treated OMW by extraction–electro-coagulation process (Fig. 1) was excellent (Table 1). It could be directly fed as influent to anaerobic reactor.

3.4. Anaerobic bio-treatment

3.4.1. Anaerobic digestion of not pre-treated OMW

The first anaerobic digester was fed with the diluted OMW (25%) at a first loading rate of $1\,g\,COD\,l^{-1}\,day^{-1}$ followed by higher loading rates. The evolutions of loading rate, biogas productivity, and methane yield during the anaerobic treatment are presented in Fig. 5A. The anaerobic filter was operated at influent OMW concentration of $29.5\,g\,COD\,l^{-1}$. Results showed that the mean COD reduction was 60% during the first period of the experiment (data not shown). The yield of methanisation of untreated OMW was higher than $0.3\,l\,CH_4\,g^{-1}$ COD introduced at low loading rates. However, since the 26th day, when the loading rate reached a mean of $3.5\,g\,COD\,l^{-1}\,day^{-1}$ (Fig. 5Aa), a decrease in the biogas productivity and methane yield were observed (Fig. 5Ab and Ac). This toxicity was accompanied by

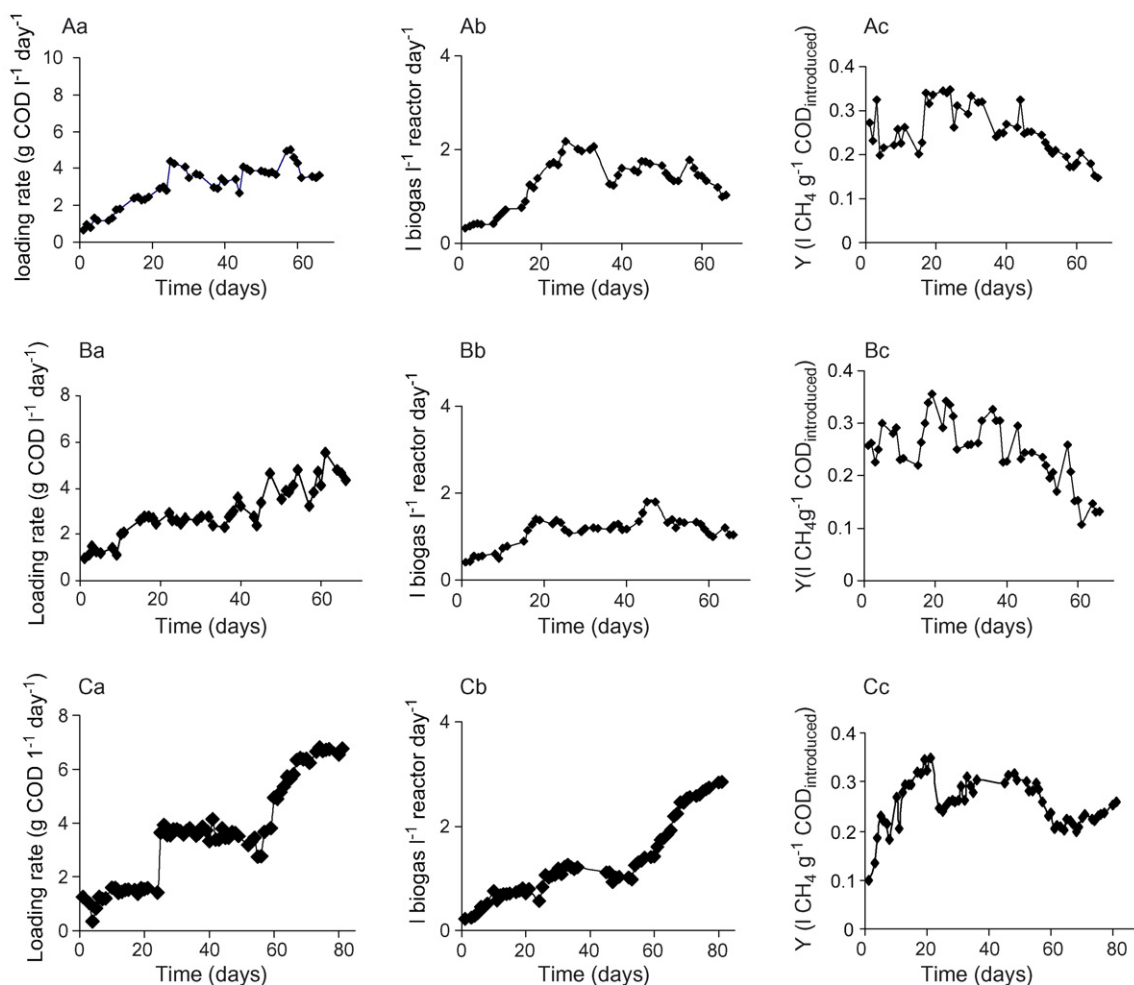Fig. 5. Evolution of the loading rates ($g\,COD\,l^{-1}$ reactor day^{-1}) (a), biogas productivity (l biogas l^{-1} reactor day^{-1}) (b) and methane yield ($l\,CH_4\,g^{-1}$ COD$_{introduced}$) (c) during anaerobic digestion of raw OMW (A), exhausted fraction (B) and exhausted fraction pre-treated by electro-coagulation (C).

a pH decrease in the three levels of the reactors and an accumulation of the VFA (data not shown). This test of the anaerobic digestion of untreated OMW by an 8 years OMW-acclimated consortium will serve as a control for comparing the efficiency of the solvent extraction pre-treatment and electro-coagulation in the detoxification of this effluent. Several authors reported that the inhibition of the methanisation of crude OMW occurred at mean loading rates of 1.5 g of $COD \, l^{-1} \, day^{-1}$ [28].

Previous reports concluded that the presence of phenolic compounds in OMW inhibit the growth of certain microorganisms, particularly bacteria, and is the major cause, together with fatty acids, for the methanogenic toxicity of OMW [29]. In previous experiments on the anaerobic digestion of unmodified OMW, many problems such as the high toxicity, low biodegradability and the acidification of reactors were studied [30]. A pre-treatment step was deemed necessary for decreasing the organic load and the phenolic compounds, potential inhibitors of methanogenesis.

3.4.2. Anaerobic digestion of the exhausted fraction of OMW

The second anaerobic filter was fed with diluted (25%) exhausted OMW fraction at a first loading rate of $1 \, g \, COD \, l^{-1} \, day^{-1}$ followed by higher loading rates. At low loading rates from 1 to $3.5 \, g \, COD \, l^{-1} \, day^{-1}$, the biogas productivity increased with increasing the loading rate which resulted in high methanisation yield ($0.31 \, CH_4 \, g^{-1}$ COD introduced) (Fig. 5B). At this period of fermentation, the inlet and outlet concentrations of COD were 24.5 and $8.5 \, g \, l^{-1}$, respectively. However, increasing the loading rate to more than $3.5 \, g \, COD \, l^{-1} \, day^{-1}$ resulted in a decrease of the yield which reached $0.123 \, l \, CH_4 \, g^{-1}$ COD introduced in the 66th day, suggesting an inhibition phenomenon (Fig. 5Bc).

Anaerobic digestion is a complex process consisting of a series of microbial transformation of organic materials to methane and VFA such as acetate, propionate, butyrate, *iso*-butyrate, valerate and *iso*valerate. These VFA have long been recognized as the most important intermediates in the anaerobic process and have been proposed as a control parameter [16,31]. Therefore, changes in VFA concentration can be in response to variation in temperature, organic loading rates or the presence of toxicants. The dynamics of VFA production and pH measurements at the bottom (A), the medium (B) and the top (C)

of the reactor are shown in Fig. 6. Fig. 6a showed that VFA concentrations were very low at the beginning of the loading. From the 20th day, VFA concentrations rose in the point A and gradually increased in the points B and C. This increasing of VFA is well correlated with the decrease of pH at the three levels (Fig. 6b) and therefore with the acidification of the reactor. This phenomenon appeared at the period of high loading rate where a significant decrease of biogas and methane production was shown. We can conclude then, that pre-treatment of OMW by ethyl acetate extraction does not improve the anaerobic post-treatment. In this case, we can note that methanogenesis activity could be mainly inhibited by the presence of the HMM compounds and residual lipids. These results confirmed the findings of Sayadi et al. [20] who showed that the HMM fraction (isolated from crude OMW) provoked unexpectedly an inhibition of the methanisation.

We conclude that the ethyl acetate extraction decreased OMW monomeric phenols by more than 90%. This did not result, however, in a corresponding reduction in biotoxicity, as tested by methanisation of the exhausted fraction of OMW in an anaerobic filter. On the basis of these results, a pre-treatment step before anaerobic digestion of the exhausted fraction was proposed. Recently, interest has been focused on the electro-chemical processes such as electro-oxidation, electro-coagulation and electro-flotation. Among these methods, electro-coagulation pre-treatment of OMW before anaerobic digestion has shown important results [27].

3.4.3. Improvement of the anaerobic digestion of exhausted OMW fraction by electro-coagulation

The anaerobic digester was fed with undiluted pre-treated OMW at a first loading rate of $1 \, g \, COD \, l^{-1} \, day^{-1}$ followed by higher loading rates. The evolutions of loading rate, biogas productivity, and methane yield during the anaerobic treatment are presented in Fig. 5C. The reactor was operated at influent OMW concentration of $38.5 \, g \, COD \, l^{-1}$ (mean value). Results showed that the mean COD reduction was 89% during the operation of the anaerobic digestion (data not shown). Fig. 5C shows that the biogas productivity increased with increasing the loading rate. The percentage of methane in the biogas produced by the anaerobic filter was found to be in a range between 65 and 70% (data not shown), with an average methane yield of about $0.251 \, CH_4 \, g^{-1}$ COD introduced. Fig. 5C shows that, at the highest loading rates
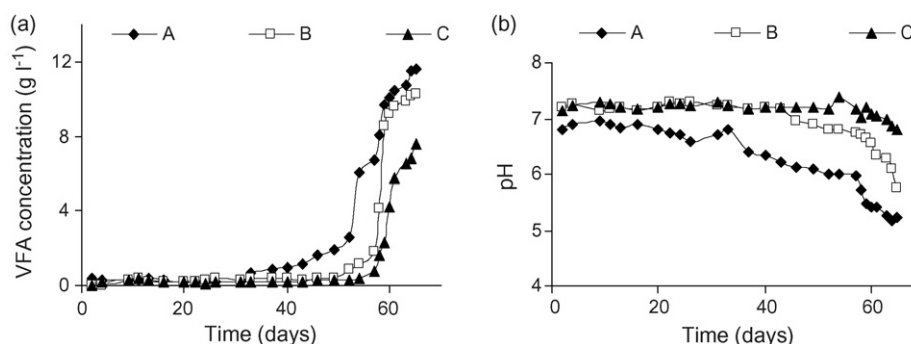

Fig. 6. VFA concentration (a) and pH (b) Evolutions in the anaerobic filter during the methanisation of the OMW exhausted fraction (A: bottom of the reactor, B: middle of the reactor, C: top of the reactor).

$(6.5\text{--}7.5\,\mathrm{g\,COD\,l^{-1}\,day^{-1}})$, the yields obtained were approximately $0.25\,\mathrm{l}$ of $CH_4\,g^{-1}$ of COD introduced. The higher values of yields $(0.30\text{--}0.34\,\mathrm{l}\,CH_4\,g^{-1}\,\text{COD introduced})$ were obtained with loading rates less than $4.5\,\mathrm{g\,COD\,l^{-1}\,day^{-1}}$. The increase of loading rate from 4 to $7.5\,\mathrm{g\,COD\,l^{-1}\,day^{-1}}$ shows a progressive increase of biogas productivity. The $3\,\mathrm{l}$ anaerobic filter produced more than $9\,\mathrm{l}$ of biogas per day at a loading rate of $7\,\mathrm{g\,COD\,l^{-1}\,day^{-1}}$. The bio-methanisation process was found to be stable during all the period of operation without any toxicity phenomenon. This was noted by the stability of pH at optimum value of 7.2 and the low concentrations of VFA even at the higher loading rates at the different levels of the reactor (data not shown).

4. Conclusion

In this study, liquid–liquid extraction of OMW using ethyl acetate was investigated as a pre-treatment before biological treatment. This pre-treatment has two advantages: profitable use of OMW phenols as antioxidant and a toxicity decrease of the wastewater.

GC–MS analysis noted that several free phenols could be recovered from OMW by a simple liquid–liquid extraction process. Phenolic extract was composed of hydroxytyrosol as major compound, and also tyrosol, cafeic acid, *para*-coumaric acid and ferulic acid in minor concentrations. Indeed, this OMW extract might be a natural source of useful substances.

The results of COD and GC–MS analysis demonstrate that diluted exhausted fraction was partly biodegradable in batch aerobic digestion. The methanisation of the exhausted fraction of OMW showed high performance at loading rates of $1\text{--}3.5\,\mathrm{g\,COD\,l^{-1}\,day^{-1}}$ but when the loading rate was increased, a disruption of the methanisation was observed. The methanogenesis activity could be mainly inhibited by the presence of HMM compounds and residual lipids. Electro-coagulation of the exhausted fraction was suggested before anaerobic digestion for decreasing the organic load and the HMM phenolic compounds. Results show that the removal of HMM phenolic compounds using electro-coagulation contributes significantly in increasing the efficiency of the anaerobic digestion.

Acknowledgements

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