Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2018.21916

112 (2018) 232–241
April

Valuable phenolic compounds recovery from olive mill wastewater streams by means of cooling crystallization techniques

S.S. Kontos[a,b], I.S. Ioannou[a,b], P.G. Koutsoukos[a,b], C.A. Paraskeva[a,b,*]

[a]*Department of Chemical Engineering, University of Patras, Rion, Patras, GR 26504, Greece, Tel. +30 2610 997252; Fax: +30 2610 997574; emails: takisp@chemeng.upatras.gr (C.A. Paraskeva), spyretos@chemeng.upatras.gr (S.S. Kontos), iasonasce@gmail.com (I.S. Ioannou), pgk@chemeng.upatras.gr (P.G. Koutsoukos)*
[b]*Institute of Chemical Engineering Sciences, Foundation for Research and Technology, Hellas (FORTH/ICE-HT), Stadiou Str., Platani, Patras, GR 26504, Greece*

Received 20 October 2017; Accepted 13 January 2018

ABSTRACT

Olive mill wastewater (OMW), produced during the olive oil extraction process, is a hardly degradable by-product characterized by significant high concentrations of organic compounds and complex mixtures of phenolic compounds. The treatment of OMW is considered as a very difficult task because phenolic content is highly problematic for aerobic or anaerobic treatment and at present there is no unique and affordable process for OMW treatment. On the other hand, phenolic compounds in OMW are considered as high added value constituents and their isolation, purification and recovery is related with the higher efficiency in the management and valorization of the olive mills effluents. Physicochemical treatment methods, based on membrane filtration suggested recently by several researchers, proved successful in the fractionation and isolation of phenolic compounds in the concentrated streams of either nanofiltration or reverse osmosis (RO) units. Unfortunately, rich in phenolics streams have a high content of monosaccharides or disaccharides since the molecular size of the sugars is comparable with the respective of individual phenolic compounds. The present work is associated with the development of a more sophisticated treatment process of wastewater through the application of membrane filtration, cooling crystallization and melt crystallization to enhance the isolation, recovery and purification of phenolic compounds when present at high concentrations. The experimental study was done in synthetic media simulating the RO concentrates of OMW. Glucose and most part of OMW polyphenols (including tyrosol, ferulic acid [FA] and trans-cinnamic acid [TCA]) served as model compounds for sugars and phenolic compounds, respectively. It was found that in the case of mixtures, high recovery yields of FA, tyrosol and TCA were achieved.

Keywords: Olive mill wastewaters; Phenolic compounds; Membrane filtration; Cooling crystallization; Modified operational conditions

1. Introduction

Olive oil production is associated with the coproduction of large amount of residual wastewater, known as olive mill wastewater (OMW), due to the addition of high quantities of water during olive oil processing. The efficient treatment and management of the coproduced OMW is a matter of paramount importance as its uncontrolled disposal into aquatic receptors is responsible for adverse environmental effects. As a matter of fact, it is imperative for all Mediterranean countries, engaged with olive oil production, to develop viable, cost effective treatment methods of the OMW effluents [1]. Anaerobic or aerobic digestion which is well known processes for the effective treatment of solutions with high organic content is inhibited in OMW treatment because of the presence of phenolic compounds [2,3]. New trends in treating OMW suggest phenolic compounds removal from OMW before further

* Corresponding author.

Presented at the 5th International Conference on Sustainable Solid Waste Management (ATHENS 2017), 21–24 June 2017, Athens, Greece.

treatment [4–6]. Polyphenols are high added value components of OMW and their potential recovery in pure form is important because of their potential use in food industry, cosmetics and pharmaceuticals [7–9]. In the present work, the combination of different physicochemical treatment methods is proposed for the investigation of the development of an integrated exploitation system for OMW. More specifically, it is suggested that physicochemical treatment methods, including membrane filtration, evaporation, cooling crystallization and melt crystallization may be combined into an integrated OMW treatment methodology [10]. Membrane filtration has been extensively applied for the purification of OMW [11–15]. During this process, the separation of various components takes place, based on the membrane's molecular weight cut-off. Paraskeva et al. [16,17] carried out a detailed parametric study using a sequence of membrane units for the investigation of the possibility of complete fractionation of OMW. The application of ultrafiltration (UF) step achieved the separation of high molecular weight components, whereas the phenolic compounds were successfully fractionated at the nanofiltration (NF) step. The concentrated solutions of NF were rich in phenolic compounds and were further tested in hydroponic systems and seemed to favor the development of native plants, while exhibiting excellent herbicide activity. The financial consideration of the proposed membrane scheme is discussed in the work of Arvaniti et al. [18]. Stoller [19] developed a simulation code of NF membrane module for the purification of OMW based on the corresponding critical flux concept. The proposed method appears to give very promising results and is almost fouling-free over a very long operational period. Ochando-Pulido et al. [20] proposed a combination of physicochemical methods for the effective treatment of OMW produced in two or three phase decanter processes. Initially, they used a pretreatment process including pH–temperature controlled flocculation, followed by UV/TiO_2 photocatalysis with ferromagnetic core nanoparticles. The application of the pretreatment process seemed to be very effective for both extraction processes. As a next treatment step, a series of UF, NF and reverse osmosis (RO) polymeric membrane modules were subsequently used for the purification of the effluent. Zagklis and Paraskeva [21] applied a combined system of membrane filtration and rotary evaporation for the exploitation of OMW and defect wine resulting in solutions enriched in simple, low molecular weight compounds (phenols and simple carbohydrates) from the initial wastes. Further isolation and purification of phenolic compounds were achieved in the work of Zagklis et al. [22]. Purification of phenolic compounds was done by adsorption/desorption from resins (non-ionic XAD4, XAD16 and XAD7HP resins). The final results were very encouraging leading to higher recovery yields of phenolic compounds (378 g L^{-1}) compared with the content of the sugars (293 g L^{-1}).

The increasing need for further recovery of pure phenolic compounds in crystalline form, led to the implementation of cooling crystallization, in which the various components may be separated from the respective aqueous fluid on the basis of their solubilities over the temperature range tested. Recently, Kontos et al. [23] investigated the possibility of extracting purified polyphenols from OMW by cooling crystallization, using two model phenolic compounds, typically found in OMW, namely trans-cinnamic acid (TCA) and ferulic acid (FA). The thickness of the layers, deposited on the cooled fin as a function of crystallization time, was measured experimentally and modeled successfully [1]. Further investigation of the cooling crystallization of phenols was done in model solutions containing FA and TCA. The final recovery yields showed that the potential separation of phenols by cooling crystallization is feasible and promising.

In the present work, the main objective was the application of cooling crystallization as an intermediate treatment process for the separation of phenolic compounds from sugars present in pretreated OMW streams. Cooling crystallization experiments took place in the presence of phenolic compounds and sugars from aqueous solutions at a temperature of 60°C. Specifically, mixtures of FA, TCA, tyrosol and glucose were used as model compounds for the investigation of their recovery with the simultaneous reduction of the glucose content entrapped in the recovered solid.

As a next step, the development of an integrated management process for OMW using a combination of different physicochemical techniques is proposed. The application of membrane technology was proposed for the fractionation of simple phenolic compounds from OMW on the basis of their different molecular weight size. Next, the application of vacuum distillation was applied for the removal of the solvent in order to increase the final saturation with respect to each phenolic compound. Cooling crystallization was suggested as the next in series treatment step. The quantitative results of the present work aimed at the application of cooling crystallization as an intermediate treatment step, before the application of melt crystallization, for the potential further separation of phenolic compounds from the respective sugars of similar molecular weight size based on their different freezing points.

The current work is a first step for the development of a satisfactory integrated process for the recovery of phenolic compounds by cooling crystallization, which could possibly be scaled up at a later stage for industrial application.

2. Materials and methods

2.1. Determination of TCA, FA, tyrosol and glucose in the solid crystal layer

Synthetic crystalline phenolic compounds, TCA, FA and tyrosol were obtained from Sigma-Aldrich (St. Louis, USA). Glucose was purchased from Carlo Erba Reagents (Milan, Italy). The quantitative analysis in the crystallization experiments including FA, TCA and glucose was performed with the combination of titration and spectroscopic analyses. Specifically, FA was measured via Folin-Ciocalteu method at 760 nm [24]. Glucose was determined spectrophotometrically at 525 nm using L-tryptophan reagent [25]. The quantitative analysis of the concentrations of the crystalline TCA was measured by titrations with 0.01 N NaOH, using ethanol as solvent [26]. Moreover, quantitative analysis of FA, TCA, tyrosol and glucose was done by high performance liquid chromatography (HPLC). The HPLC (Alliance 2695 supplied from WATERS) was equipped with an UV/Vis Diode Array detector (2996 PDA Detector). The separation of each compound of the solid layer was carried out using a chromatographic column Prodigy 5u OPJ3 100A with dimensions 250 × 4.6 mm and particle size of 5 μm from the Phenomenex.

2.2. Crystallization experiments

The apparatus used for the crystallization experiments has been recently described in detail elsewhere [23]. Briefly, a double wall Pyrex® glass vessel of inner diameter 5 cm and active volume 50 mL was used as crystallization reactor. The temperature at the walls of the vessel was kept constant by circulating fluid from a thermostat. The circulation of cold water, in the inner side of the cylinder, ensured the application of constant cold temperature conditions at the cooled fin (Ø 25 mm, L = 120 mm). At the beginning the temperature of the vessel was raised to 60°C under stirring with a magnetic stirrer to ensure complete dissolution of each of the test compound. Next, following equilibration at 60°C, a cooled fin was immersed in the hot solution. After different time intervals from the onset of crystallization, the cylinder was removed and the characterization and measurements of the formed solid layer was done by measuring its thickness and analyzing its components quantitatively as already described.

3. Results and discussion

3.1. Effect of supersaturation on the onset of crystallization on the cooled fin

The prerequisite for nucleation and crystal growth was the establishment of supersaturation for each compound present in the solution. In the present work, the phenolic compounds tested were FA, TCA and tyrosol. Glucose was used as the model carbohydrate component. The solubility of FA [27] and TCA [1] in water was measured and presented in detail elsewhere. Tyrosol and glucose solubility dependence on temperature in the range of the present work conditions has been presented elsewhere [28]. The saturation concentration is a function of temperature. The solubility of FA in water is higher than the respective TCA. The solubility of tyrosol was one order of magnitude higher in comparison with the solubility of FA and TCA over the test temperature range.

In the following set of experiments, the initial concentration of TCA and FA in the crystallizer was selected approximately equal to the saturation concentration for the temperature range 55°C–60°C. Specifically, the initial concentration of TCA and FA was 1 and 3 g L^{-1}, respectively, whereas the initial concentration of glucose was 69 g L^{-1}. The initial concentration of tyrosol in the crystallizer varied over the range of 2, 20 and 65 g L^{-1} in the presence of the rest of the test compounds, in order to examine their effect on the recovery of the solid. For solution temperature values above 60°C, the phenolic compounds were completely dissolved in water, while glucose concentration remained one order of magnitude below equilibrium concentration. The experiments were performed at temperature values of circulating fluid equal to T_{cold} = 5°C, 10°C and 15°C. The saturation concentration values and the initial concentrations of the test compounds for the tested temperature values of the cooled fin are presented in Table 1.

The heat transfer, induced from the walls of the vessel to the cooled fin, was the driving force for the formation of a temperature distribution in the crystallizer rendering the crystallization of the desired phenolic compounds thermodynamically feasible. TCA and FA initial concentrations selected, ensured the application of a large concentration difference, $\Delta c = c_L - c_{sat}$, in the system sufficient for the initiation of nucleation and crystal growth in the cooled fin, immediately past the immersion of the cooled surface in the hot solution.

Table 1
Initial concentration and saturation concentration values (g L^{-1}) of the tested compounds at temperature conditions 5°C, 15°C and 60°C

Compound	Initial concentration (g L^{-1})	Saturation concentration (g L^{-1})		
		5°C	15°C	60°C
FA	3	0.35	0.728	3.07
TCA	1	0.16	0.17	1.39
Tyrosol	Varied (2, 20 and 65)	24.8	51.2	1,326
Glucose	69	554.9	589.1	752.1

3.2. Crystallization of FA and TCA in the presence of glucose

Two series of experiments were done to test the recovery of FA and TCA from aqueous solutions in the presence of high glucose concentrations. The target in this study was to isolate FA and TCA minimizing the potential of coprecipitation of glucose in the matrix of the solid formed on the cold metallic surface. In the first series, crystallization took place under constant temperature of the circulating fluid (5°C) while in the second series of experiments, the temperature was gradually reduced from 15°C to 10°C (0.3°C min^{-1} for time interval of 15 min for the adjustment of the temperature of the cold water) and finally to 5°C (0.25°C min^{-1} for time interval of 20 min) during the test run. Fig. 1 shows pictures of the progress of crystal growth on the cooled fin for the different applied temperature settings of the circulating fluid past 10 min, 1 h and 10 h from the onset of crystallization.

In Fig. 1(b), the time intervals of 10 min and 1 h from the immersion of the cooled fin correspond to temperature setting of the cold circulating fluid equal to 15°C, whereas past the removal of the cooled fin at time interval of 10 h, the crystal layer was formed at cold fluid temperature of 5°C.

3.2.1. Crystallization of mixtures of FA, TCA and glucose (total duration of 10 h, at constant temperature of the cold (5°C) and hot water bath (60°C)

Stock solutions of FA and TCA in water in the presence of glucose were prepared, and subsequently were heated to an initial solution temperature of 60°C. The concentrations of phenols and sugars, according to literature reports for the liquid effluents of olive mills, were adjusted so that their ratio was ca. 1:17 [21]. Past the removal of the fin from the hot solution, the quantitative analysis of each compound, deposited on the fin, followed as shown in Figs. 2(a) and (b).

As may be seen in Fig. 2(a), a substantial reduction in the mass fraction of glucose was achieved. Specifically, from 95% of glucose present in the initial solid, the recovered glucose was sufficiently reduced to 50% at the initial stages of crystallization. Past 10 h from the onset of crystallization, a further decrease in the mass fraction glucose was achieved reaching ca. 40%. It is interesting to note that the mass fraction of

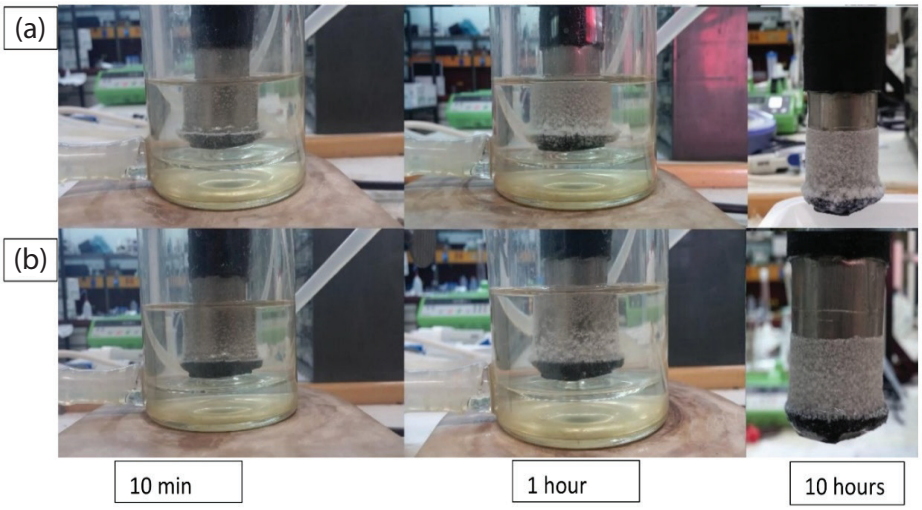

Fig. 1. Evolution of the crystal layer deposited on the cold fin as a function of the crystallization time for (a) constant temperature (5°C) and (b) different temperature settings of the circulating fluid (from 15°C to 10°C and finally to 5°C).

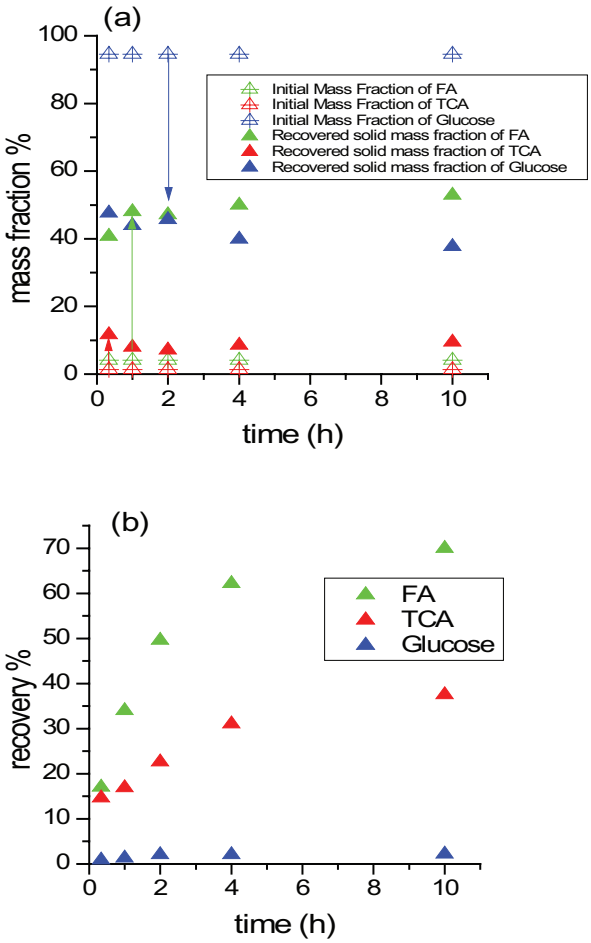

Fig. 2. (a) Mass fraction and (b) % recovery of FA, TCA and glucose precipitated on the cooled fin as a function of time for different time intervals from the onset of crystallization at constant refrigerant temperature (arrows show the increase of the mass fraction of FA and TCA and the reduction in the mass fraction of glucose regarding the recovered solid).

glucose was higher in the first 30 min of the operation, while it decreased with the progress of crystallization. Similar results have also been reported in the work of Parisi and Chianese [29] who showed that impurities were included in the solid layer at the initial stages of crystallization. Concerning the mass fraction of the two phenolic compounds, a substantial increase in the recovered solid was observed. From the initial 4% and 1%, mass fractions of 50% and 10% were obtained for FA and TCA, respectively. The satisfactory results of the composition of the recovered solid were accompanied by high recovery yields for FA and relatively high for TCA (Fig. 2(b)). Similar cooling crystallization experiments of mixtures of FA and TCA were performed in the absence of glucose and similar % recovery yields were achieved for both phenolic compounds, as reported in the work by Kontos et al. [23].

As may be seen in Fig. 2(b), glucose recovery was very low because, the initial glucose concentration was sufficiently lower than the corresponding saturation concentration [28]. The low recovery was attributed to the bulk solution retained in the phenolic recovery layer. In particular, from the 3.450 g of glucose, in the initial solid, only 75 mg were found in the final crystal layer deposit. Furthermore, as may be seen in Fig. 2(b), at the initial stages of crystallization, the composition of TCA in the crystalline layer was rather high. However, the evolution of crystallization led to a decrease of the TCA mass fraction and of the recovery rate. This may be attributed to the temperature of the fin. Initially, the temperature of the cooled fin was equal to the temperature of the circulating fluid (5°C). Therefore, the temperature at the close vicinity of the fin increased gradually, because of the formation of solid on the cylinder, resulting in respective changes of the saturation concentration.

3.2.2. Crystallization of mixtures of FA, TCA and glucose for 10 h at different temperatures of the circulating fluid

In this series of experiments, the only difference, was the alteration of the temperature of the circulating fluid which gradually changed from 15°C to 10°C past 4 h and finally

to 5°C past 8 h from the onset of crystallization. The experiments aimed at the investigation of the possibility to increase purity of the crystalline layer with respect to its content in phenolic compounds. The total mass of the recovered solid was measured ca. 160 mg. Table 2 shows the temperature alteration of the circulating fluid over the crystallization time of the experiment.

As may be seen in Table 2, there was a delay from the setting of the temperature in the bath till the establishment of the adjustable temperature in the coolant. The mass fraction and the recovery of FA, TCA and the entrapped glucose deposited on the fin are summarized in Fig. 3.

As may be seen in Fig. 3(a), the mass fraction of glucose in the recovered solid was further reduced (up to 10%) in comparison with the reduction obtained at constant temperature settings of the circulating fluid at 5°C (Fig. 2(a)). Comparison of Figs. 2(b) and 3(b), suggested that the change of the coolant temperature, did not significantly affect FA recovery. For the case of TCA, it was shown that at the initial stages of crystallization, TCA recovery rate increased rapidly, reaching a plateau past 1 h from the onset of crystallization. Setting of the circulating fluid at 10°C, resulted in higher recovery of TCA, ca. 30%. Similar behavior was observed in the third stage of operation (temperature setting of the coolant at 5°C). An interesting observation for both sets of experiments was the fact that the % final recovery of FA and TCA was of the same order of magnitude. This result may be attributed to the fact that the final setting temperature of the cooling fluid ensured the same thermodynamically possible recovery for the examined phenolic compounds. In Fig. 4, the mass fraction and the recovery rate of glucose entrapped in the solid layer are presented for different periods from the immersion of the cooled fin in the hot solutions.

As shown in Fig. 4(a), the average mass fraction of glucose at varied temperature conditions of the coolant was ca. 28% of the total recovered solid, significantly lower than the 95% of the initial solid and lower than the recovery percentage of glucose (42%) at constant temperature conditions of the refrigerant (5°C). In particular, from the 75 mg entrapped in the first series of experiments (temperature of the coolant constant at 5°C), the final glucose mass was significantly lower (45 mg, varied temperature settings of the cold fluid).

The main objective of this series of crystallization experiments was the investigation of the efficiency of cooling crystallization for the recovery and isolation of high added value products present in the effluent. From the experimental study with synthetic phenolic compounds, it was observed that the selective recovery of phenolic compounds was feasible from aqueous solutions in the presence of high initial concentration levels of glucose. The initial concentration of phenols and sugars was in agreement with reports given by Zagklis and Paraskeva [21] where the ratio of phenols over sugars in OMW was found ca. 1:17. High recovery yields of phenolic compounds (70% for FA and 40% for TCA) were achieved in the present work. Furthermore, the mass fraction of the entrapped glucose in the solid layer was measured around ca. 10% lower in the experiments at controlled temperature reduction of the circulating fluid. The most important result was the fact that from an initial ratio of phenolic compounds over glucose 1:17, at the end of crystallization, the ratio reached the value of 2.5:1. Thus, the separation of phenolic compounds from sugars was feasible provided that a suitable supersaturation was established for each compound in the examined temperature settings.

Table 2
Summary table of temperature change of the cooling fluid

Operational time (h)	Temperature setting of the cooling fluid (°C)	Estimated time for the temperature change of the coolant (min)
0	15	–
4	10	15
8	15	20

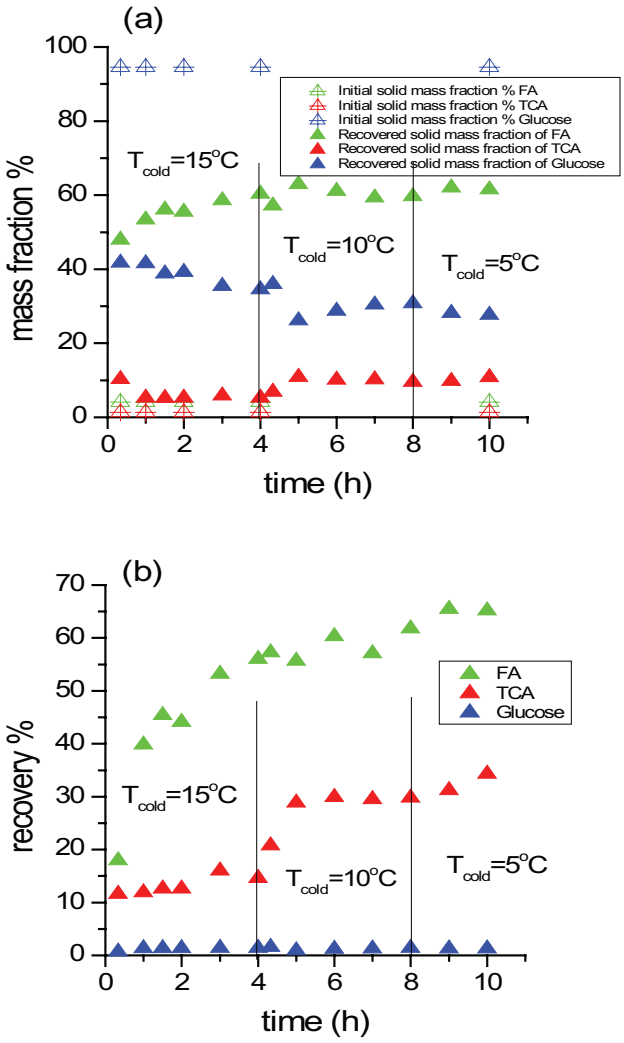

Fig. 3. (a) Mass fraction and (b) % recovery of FA, TCA and glucose deposited on the cooled fin as a function of crystallization time for different settings of the temperature of the circulating fluid.

3.3. Crystallization of FA, TCA and tyrosol in the presence of high concentration levels of glucose

For the investigation of the recovery of polyphenols FA, TCA and tyrosol from supersaturated solutions in the

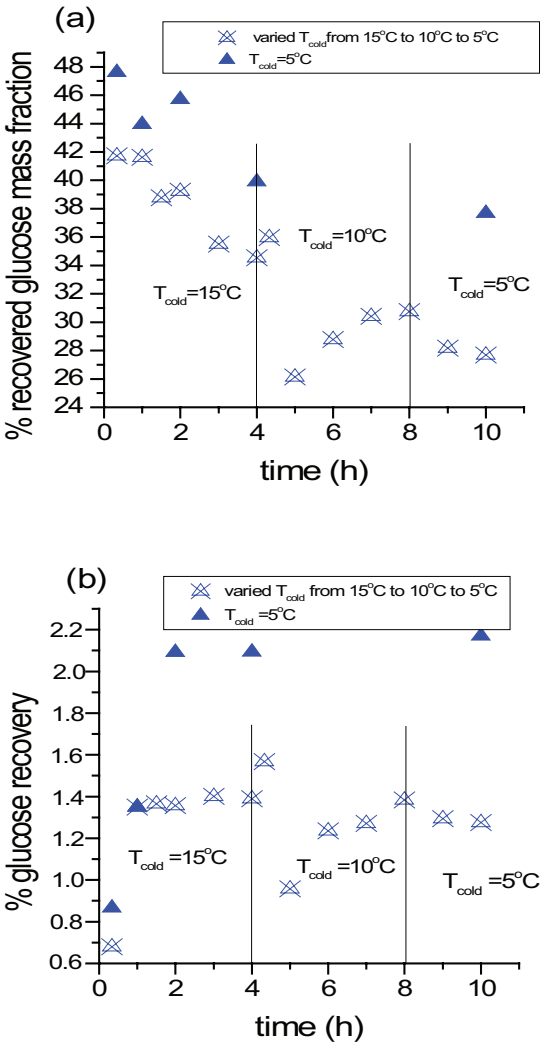

Fig. 4. Comparative diagram of (a) mass fraction and (b) % recovery of glucose entrapped in the crystallized solid, as a function of the crystallization time.

presence of glucose at high initial concentration levels, cooling crystallization experiments were performed at different initial concentrations of tyrosol and at different temperature of the hot vessel.

This experimental study was done because, as reported by Kontos et al. [1], the concentration levels of tyrosol present in OMW are significantly higher in comparison with the concentrations of FA and TCA. Moreover, the recovery of tyrosol is very important, as it is one of the two main phenolic compounds present in OMW and is also characterized by high added value with potential applications in the treatment of a number of pathologic situations [30].

3.3.1. Crystallization of mixtures of FA, TCA, tyrosol (2 g L^{-1}) and glucose of total duration of 10 h at constant temperature conditions of the hot and cold circulating fluids

In the following set of experiments, crystallization of mixtures of FA, TCA, tyrosol and glucose were carried out for the investigation of their recovery from supercooled solutions. The crystallization conditions of the hot (60°C) and cold (5°C) fluid, circulating in the vessel and the fin, respectively, were selected in order to impose a large temperature gradient in the hot solution taking into account the variation of solubility of FA, TCA, tyrosol and glucose within the examined temperature range. The solubility of tyrosol increases significantly over a narrow temperature range [27,31]. In this set of experiments, 150 mg FA, 50 mg TCA, 3,450 mg glucose and 100 mg tyrosol were dissolved in a vessel, volume totaling 50 mL. The initial concentrations of FA, TCA and glucose are summarized in Table 1. The initial concentration of tyrosol (2 g L^{-1}) was selected one order of magnitude lower than the saturation concentration. The comparative presentation of the mass fraction and the recovery percentage of FA, TCA and of the entrapped glucose precipitated on the fin in the presence and absence of tyrosol are summarized in Fig. 5 and Table 3.

As may be seen in Fig. 5, the addition of 100 mg of tyrosol did not affect the final mass fraction and the % recovery of FA and TCA since the quantitative analysis of each compound at the end of the experimental procedure, showed similar results to those obtained in the absence of tyrosol.

As shown in Table 3, the entrapped tyrosol (1.74% at t = 10 h) and glucose (1.87% at t = 10 h) in the crystal layer was of small and similar amount for all the set of experiments despite the small fluctuations during the measurements that may be considered insignificant.

3.3.2. Crystallization of mixtures of FA, TCA, tyrosol (20 g L^{-1}) and glucose at duration of 10 h at constant temperature conditions of the hot and cold circulating fluids

As a next step, for the further investigation of the effect of tyrosol on the recovery rate of the desired components, the cooling crystallization process was investigated using higher initial concentrations of tyrosol in the fluid. More specifically, an aqueous solution of 20 g L^{-1} was introduced in the reactor, volume totaling 50 mL in which the initial concentrations of FA, TCA and glucose were the same in the previous series of experiments. The temperature conditions of the hot (60°C) and cold (5°C) fluid, circulating in the vessel and the fin, respectively, were the same as before, where lower initial concentration of tyrosol (2 g L^{-1}) was used. Next, the cooled fin was immersed in the hot solution, for 7.5 h. Crystallization on the cylinder cold surface was not observed over this period of time. This was attributed to the relatively higher concentration of tyrosol that inhibited the transport and crystallization of FA and TCA on the fin. In the next series of experiments, cooling crystallization was done at conditions in which tyrosol dissolution approaches the limits for supersaturation.

3.3.3. Crystallization of mixtures of FA, TCA, tyrosol (65 g L^{-1}) and glucose at different temperatures of the hot circulating fluid

The initial concentration of FA, TCA and glucose was the same as shown in Table 1. For the case of tyrosol, the initial concentration was substantially increased in order to examine the recovery of the phenolic compounds at conditions close to tyrosol's supersaturation values. Specifically, 3.250 g tyrosol

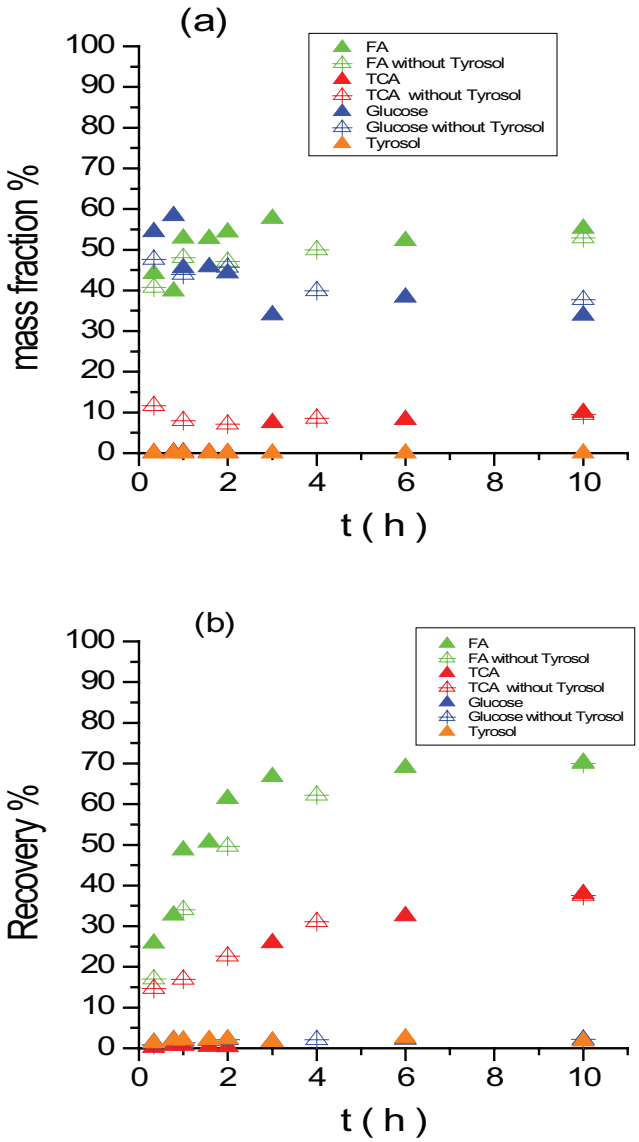

Fig. 5. (a) Mass fraction and (b) % recovery of FA, TCA, tyrosol and glucose precipitated on the cooled fin as a function of the crystallization time, at constant temperature of the circulating cold fluid (5°C).

(65 g L^{-1}), 150 mg FA (3 g L^{-1}), 50 mg TCA (1 g L^{-1}) and 3.450 g glucose (69 g L^{-1}) were added in 50 mL water in the reactor and upon their dissolution, the cooled cylinder was immersed in the hot solution. The temperature of the coolant was constant and equal to 5°C. As already shown in the present work, the crystallization of FA and TCA was inhibited in the presence of tyrosol (20 g L^{-1}) and at the same time the crystallization of tyrosol itself was not feasible because its concentration in the solution was far less than the corresponding to the desired supersaturation (65 g L^{-1}). The crystallization of tyrosol was thus investigated at different temperature values at the walls of the reactor in the range of 60°C–30°C using higher tyrosol concentrations. Specifically, two new experiments were done in two cycles, in which the temperature in the thermostated bath decreased gradually over time period exceeding 12 h. At the end of first cycle, in each experiment, the precipitated material was removed from the system (first batch) and the cooled surface was immersed again in the solution, set at lower temperature, of 45°C–55°C, in order to increase the recovery of phenolic compounds. By the end of the second cycle the cooled cylinder was removed again and the mass of the material deposited (second batch) was measured.

As may be seen in Fig. 6(a), during the first cooling cycle (high initial concentrations of tyrosol, 65 g L^{-1}), the initial temperature was 60°C. In the second cycle, where a part of the tyrosol was already removed in first cycle (first batch), a new lower initial temperature at 45°C was set for the beginning of the study of the precipitation on the cooled surface. Similar changes in the temperature profiles were used in the second series of experiments, in which the initial temperature at the first cycle was again 60°C but at the second cycle the temperature was 55°C.

What is also interesting is the final temperature at the end of the second cycle in each experiment because, as it was stated above, the dissolution of tyrosol is lower at low temperature values. As it shown in Fig. 6(b), at the second cycle of the second experiment, the temperature was reduced to ca. 30°C, while in the first experiment the final temperature was set at ca. 35°C.

Typical pictures from the first experiment are shown in Fig. 7, where substantial mass of phenolic compounds was deposited on the cooled surface, following the completion of the second cycle, despite the fact that a part of phenols was removed in the first cycle and the solution was left with significantly lower tyrosol concentration.

Table 3
Recovered quantity of each compound for different time intervals from the onset of crystallization at constant temperature of the circulating fluid (5°C)

Operational time (h)	% Recovery FA	% Recovery TCA	% Recovery tyrosol	% Recovery glucose
0.5	25.8	0.14	1.31	1.38
0.783	32.65	0.69	2.02	2.08
1.583	48.7	0.66	2.03	1.83
2	50.6	0.38	2.06	1.91
3	61.3	0.34	2.24	2.17
6	66.74	25.92	1.64	1.7
8	69	32.5	2.5	2.2
10	70.22	37.93	1.74	1.87

The measurements of the mass of phenolic compounds and glucose in the deposited layers were done after each cycle and for the two different experimental sequences. The recovery is presented in Fig. 8. The mass fractions of the three polyphenols and glucose for both cooling crystallization experiments are shown in Figs. 9(a) and (b).

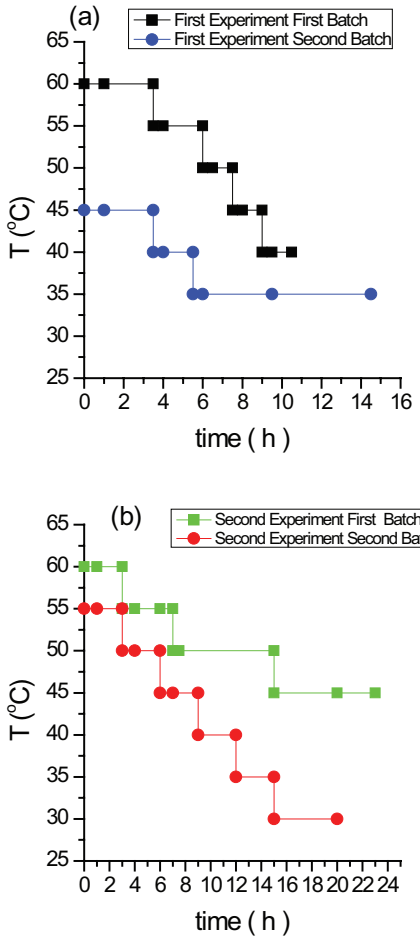

Fig. 6. Stepped variation of temperature of the thermostable bath for (a) first experiment (first and second batch) and (b) second experiment (first and second batch) as a function of crystallization time.

As shown in Fig. 8, the recovery of FA and TCA in the first cooling cycle of both experiments was almost negligible but the recovery of tyrosol was sufficiently high in the first and second cycle for both experiments. As may be seen in Fig. 8, the recovery of tyrosol per cooling cycle was satisfactory and ranged between 30% and 40% (w/w), while total recovery in both experiments approached the maximum value (63%), which could take place in the case that the temperature of the cooled fin remained constant at 5°C. Specifically, at the end of both crystallization cycles, a total recovery of 48% and 55% of tyrosol was achieved for the first and second experiment, respectively.

Surprisingly, a recovery of FA over 40% was attained in the second cycle of the second experiment and was attributed to the temperature profile used. The fact, that the total recovery of glucose was limited to less than 2%–3%, is very encouraging for obtaining pure phenolic compounds within the first two cycles. As may be seen in Fig. 8, glucose recovery rate was significantly reduced in the first cycle of crystallization of both experiments reaching 0.5%–0.6% w/w, while during the second crystallization cycle where the initiation of recovery of FA and TCA was favored, glucose recovery was similarly low as shown in Fig. 4. It is possible that during the first

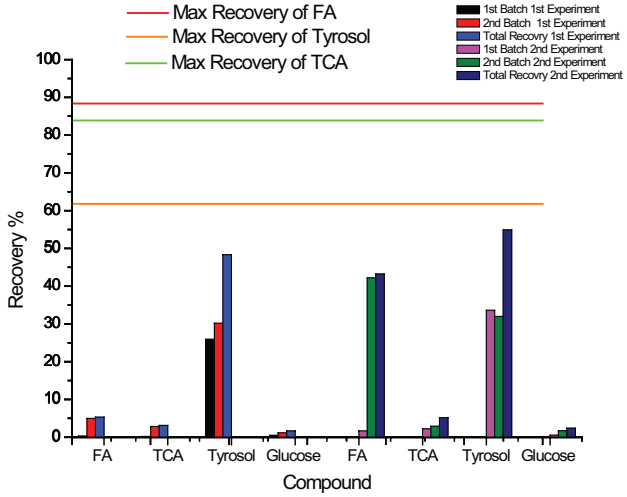

Fig. 8. % Recovery of compounds in the mixture for the two experiments.

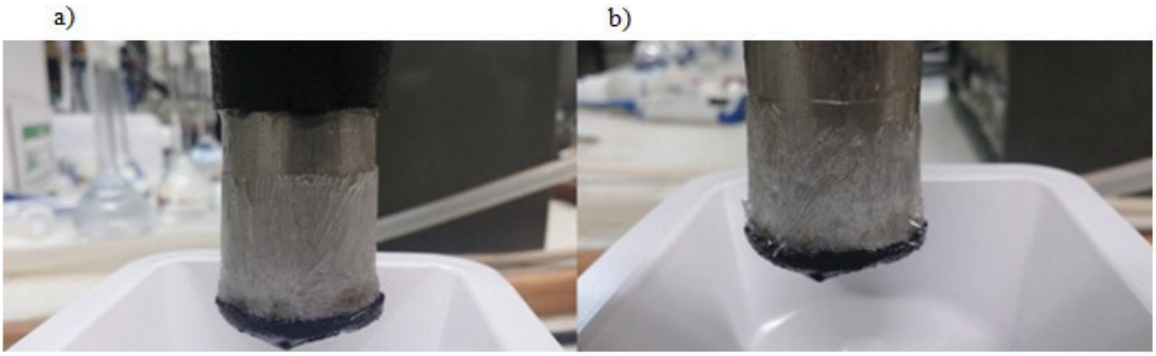

Fig. 7. Crystal layer deposited on the cooled fin for the first experiment, for (a) first cooling cycle (initial temperature of the hot water 60°C, final temperature of the hot water 40°C) and (b) second cooling cycle (initial temperature of the hot water 45°C, final temperature of the hot water 35°C).

crystallization cycle, the crystallization of tyrosol took place selectively on the cold fin. In the second cycle of crystallization, the onset of crystallization of FA and TCA resulted in higher extents of impurity inclusions.

As already shown in Fig. 5, below saturation, the addition of tyrosol did not affect the solubility of the remaining phenolic compounds, but it reduced their mass transfer rate (especially in the case of TCA) to the active growth sites of the crystal layer. In addition, as may be seen in Fig. 9, the glucose mass fraction was significantly lower in comparison with experiments in the absence of tyrosol or in the presence of low initial concentrations of tyrosol. In both experiments, the first cooling cycle resulted in the recovery of tyrosol containing very low glucose impurity concentrations. It was thus possible to attain phenolic compounds of high purity (>95%).

Finally, as may be seen in Fig. 9, the recovery achieved for tyrosol in each crystallization cycle was up to 95% (purity). On the other hand, the thermodynamically maximum recovery (shown with horizontal lines parallel to the *x*-axis in Fig. 8) was not achieved, despite the relatively long duration of the experimental process. This fact indicates that alternative ways of applying cooling crystallization need to be investigated, to reduce the operational crystallization time with the simultaneous increase of the recovered amount of the high added value products at high purity. The need for long operational time intervals should not be an inhibiting factor for further application of cooling crystallization to agroindustrial wastewater, for example, OMW, because the solution crystallization (cooling crystallization) on a cooled fin does not require constant monitoring and is characterized by low operating cost [1].

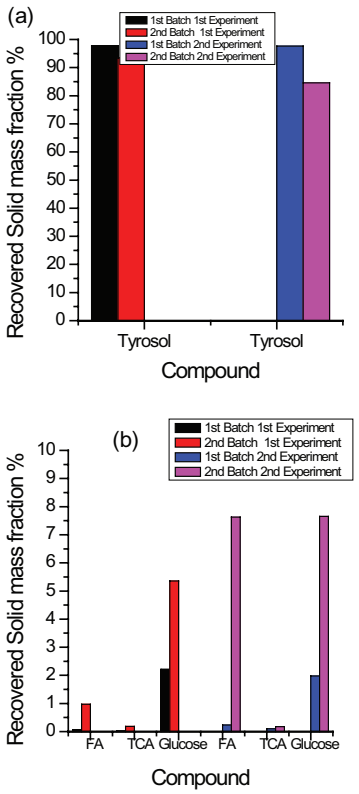

Fig. 9. % Recovery of (a) tyrosol and (b) the remaining components in the recovered solid at the end of the experimental operation.

Moreover, tyrosol is one of the most expensive phenolic compounds that appears in the OMW and as a result its recovery is of paramount importance because of its high added value [28]. As shown in this study, the alteration of the temperature setting of the hot or cold fluid may contribute to this way to render cooling crystallization as a more efficient separation process for the selective separation of phenolic compounds.

3.4. Physicochemical treatment methods applied for the treatment of OMW

The high organic and phenolic loading of OMW is the reason for its classification as a hardly degradable waste. However, efficient treatment of OMW should be combined with the extraction of high added value products, for example, phenolic compounds, present in the effluent. On the other hand, the recovery of phenolic compounds from raw OMW is not feasible without the required pretreatment of the waste as their solubility in the effluent is sufficiently lower than the concentrations needed for the onset of crystallization. Furthermore, the coexistence of sugars at high concentrations in OMW seems to be a prohibitive factor for the recovery of pure phenols. Among the available treatment methods currently applied are the physicochemical methods. In the present work, the recovery of high added value products was investigated through the combination of different physicochemical methods. Specifically, as a first treatment step, the implementation of membrane technology is suggested for the fractionation of simple phenolic compounds from OMW based on their different molecular weight compounds, using a sequence of UF, NF and RO units [22]. The application of UF step contributes to the removal of complex phenolic compounds associated with large sugars [16]. In the NF retentate, the fractionation of most of the free phenolic compounds takes place. Finally, the simple phenolic compounds and carbohydrates are successfully isolated in the RO retentate [16,22]. Further treatment of RO retentate is suggested through the application of vacuum distillation for the removal of the solvent in order to achieve a sufficiently high supersaturation with respect to each phenolic compound. For the investigation of the recovery of pure high added value phenols, the application of cooling crystallization is proposed for the separation of phenolic compounds from sugars based on solubility differences at different temperatures. As shown in the cooling crystallization experiments of the present study, the recovery and isolation of phenolic compounds from sugars are feasible rendering this particular treatment method as a satisfactory separation process for the selective recovery of high added value components [23,28]. As a final treatment step, the implementation of melt crystallization may contribute to the further isolation of phenolic compounds based on their respective freezing/melting points.

4. Conclusions

The objective of this study was the development of a combined physicochemical treatment process for the recovery of phenolic compounds from OMW with the subsequent reduction of the mass fraction of sugars in the solid layer. Cooling crystallization experiments with model phenolic compounds and sugars were done and from the % recovery, it was found

that this separation is feasible resulting in solid layers in higher purification levels in terms of phenolic compounds. Furthermore, the operational conditions affected the recovery and the purity of the final solid. Specifically, from the results of the present work, the modifications of the temperature settings of the hot and cold water bath had a beneficial effect on the purity of the final product whereas the % recovery was similar with the recovery observed at conditions of constant temperature settings.

As a final comment, it should be emphasized that the isolation of phenolic compounds from OMW, by the proposed combined physicochemical treatment process, is imperative to obtain a final solution rich in phenolic fractions. Furthermore, cooling crystallization may be the core treatment process for the development of a satisfactory model for the effective recovery phenolic compounds from sugars of similar molecular weight size as their recovery takes place based on their solubility differences in the examined temperature range.

Acknowledgment

The current study was funded by State Scholarship Foundation/IKY through the operational program 'Research Projects for Excellence IKY/Siemens', contract No 23339.

References

[1] S.S. Kontos, P.G. Koutsoukos, C.A. Paraskeva, Removal and recovery of phenolic compounds from olive mill wastewater by cooling crystallization, Chem. Eng. J., 251 (2014) 319–328.
[2] M. Niaounakis, C.P. Halvadakis, Olive Processing Waste Management: Literature Review and Patent Survey, Elsevier, Amsterdam, The Netherlands, 2006.
[3] I.N. Sgountzos, S. Pavlou, C.A. Paraskeva, A.C. Payatakes, Growth kinetics of *Pseudomonas fluorescens* in sand beds during biodegradation of phenol, Biochem. Eng. J., 30 (2006) 164–173.
[4] A. Cassano, C. Conidi, L. Giorno, E. Drioli, Fractionation of olive mill wastewaters by membrane separation techniques, J. Hazard. Mater., 248–249 (2013) 185–193.
[5] P.C. Papaphilippou, C. Yiannapas, M. Politi, V.M. Daskalaki, C. Michael, N. Kalogerakis, D. Mantzavinos, D. Fatta-Kassinos, Sequential coagulation–flocculation, solvent extraction and photo-Fenton oxidation for the valorization and treatment of olive mill effluent, Chem. Eng. J., 224 (2013) 82–88.
[6] P. Grafias, N.P. Xekoukoulotakis, D. Mantzavinos, E. Diamadopoulos, Pilot treatment of olive pomace leachate by vertical-flow constructed wetland and electrochemical oxidation: an efficient hybrid process, Water Res., 44 (2010) 2773–2780.
[7] H. Zbakh, A. El Abbassi, Potential use of olive mill wastewater in the preparation of functional beverages: a review, J. Funct. Foods, 4 (2012) 53–65.
[8] A. Agalias, P. Magiatis, A.-L. Skaltsounis, E. Mikros, A. Tsarbopoulos, E. Gikas, I. Spanos, T. Manios, A new process for the management of olive oil mill waste water and recovery of natural antioxidants, J. Agric. Food Chem., 55 (2007) 2671–2676.
[9] C.M. Galanakis, E. Tornberg, V. Gekas, Recovery and preservation of phenols from olive waste in ethanolic extracts, J. Chem. Technol. Biotechnol., 85 (2010) 1148–1155.
[10] S.S. Kontos, F.K. Katrivesis, T.C. Constantinou, C.A. Zoga, I.S. Ioannou, P.G. Koutsoukos, C.A. Paraskeva, Implementation of membrane filtration and melt crystallization for the effective treatment and valorization of olive mill wastewaters, Sep. Purif. Technol., 193 (2018) 103–111.
[11] J.M. Ochando-Pulido, S. Pimentel-Moral, V. Verardo, A. Martinez-Ferez, A focus on advanced physico-chemical processes for olive mill wastewater treatment, Sep. Purif. Technol., 179 (2017) 161–174.
[12] J.M. Ochando-Pulido, M. Stoller, Boundary flux optimization of a nanofiltration membrane module used for the treatment of olive mill wastewater from a two-phase extraction process, Sep. Purif. Technol., 130 (2014) 124–131.
[13] M. Stoller, J.M. Ochando-Pulido, Going from a critical flux concept to a threshold flux concept on membrane processes treating olive mill wastewater streams, Proc. Eng., 44 (2012) 607–608.
[14] M. Stoller, B. De Caprariis, A. Cicci, N. Verdone, M. Bravi, A. Chianese, About proper membrane process design affected by fouling by means of the analysis of measured threshold flux data, Sep. Purif. Technol., 114 (2013) 83–89.
[15] I.S. Ioannou, S.S. Kontos, P.G. Koutsoukos, C.A. Paraskeva, Mathematical modeling and experimental coupling of solution layer crystallization on a vertically cold surface, Sep. Purif. Technol., 197 (2018) 8–17.
[16] C.A. Paraskeva, V.G. Papadakis, E. Tsarouchi, D.G. Kanellopoulou, P.G. Koutsoukos, Membrane processing for olive mill wastewater fractionation, Desalination, 213 (2007) 218–229.
[17] C.A. Paraskeva, V.G. Papadakis, D.G. Kanellopoulou, P.G. Koutsoukos, K.C. Angelopoulos, Membrane filtration of olive mill wastewater and exploitation of its fractions, Water Environ. Res., 79 (2007) 421–429.
[18] E.C. Arvaniti, D.P. Zagklis, V.G. Papadakis, C.A. Paraskeva, High-added value materials production from OMW: a technical and economical optimization, Int. J. Chem. Eng., 2012 (2012) 7.
[19] M. Stoller, Effective fouling inhibition by critical flux based optimization methods on a NF membrane module for olive mill wastewater treatment, Chem. Eng. J., 168 (2011) 1140–1148.
[20] J. Ochando-Pulido, G. Hodaifa, M. Victor-Ortega, S. Rodriguez-Vives, A. Martinez-Ferez, Effective treatment of olive mill effluents from two-phase and three-phase extraction processes by batch membranes in series operation upon threshold conditions, J. Hazard. Mater., 263 (2013) 168–176.
[21] D.P. Zagklis, C.A. Paraskeva, Membrane filtration of agro-industrial wastewaters and isolation of organic compounds with high added values, Water Sci. Technol., 69 (2014) 202–207.
[22] D.P. Zagklis, A.I. Vavouraki, M.E. Kornaros, C.A. Paraskeva, Purification of olive mill wastewater phenols through membrane filtration and resin adsorption/desorption, J. Hazard. Mater., 285 (2015) 69–76.
[23] S.S. Kontos, P.G. Koutsoukos, C.A. Paraskeva, Application of combined physicochemical techniques for the efficient treatment of olive mill wastewaters, Desal. Wat. Treat., 57 (2016) 17051–17060.
[24] P. Waterman, S. Mole, Analysis of Plant Phenolic Metabolites, Blackwell Scientific Publications, Oxford, 1994.
[25] B. Josefsson, Rapid Spectrophotometric Determination of Total Carbohydrates, K. Grasshoff, M. Ehrhardt, K. Kremling, Eds., Methods of Seawater Analysis, Verlag Chemie GmbH, 1983, 340–342.
[26] W.C. Johnson, A.H.A. Abbott, W.C. Wake, M.E. Dalziel, B. Atkinson, Abstracts of papers published in other journals. Organic, Analyst, 74 (1949) 467–473.
[27] F.L. Mota, A.J. Queimada, S.P. Pinho, E.A. Macedo, Aqueous solubility of some natural phenolic compounds, Ind. Eng. Chem. Res., 47 (2008) 5182–5189.
[28] S.S. Kontos, I.C. Iakovides, P.G. Koutsoukos, C.A. Paraskeva, Isolation of purified high added value products from Olive Mill Wastewater streams through the implementation of membrane technology and cooling crystallization process, Chem. Eng. Trans., 47 (2016) 337–342.
[29] M. Parisi, A. Chianese, The crystal layer growth from a well-mixed melt, Chem. Eng. Sci., 56 (2001) 4245–4256.
[30] D. Vauzour, G. Corona, J.P.E. Spencer, Caffeic acid, tyrosol and p-coumaric acid are potent inhibitors of 5-S-cysteinyl-dopamine induced neurotoxicity, Arch. Biochem. Biophys., 501 (2010) 106–111.
[31] A.J. Queimada, F.L. Mota, S.P. Pinho, E.A. Macedo, Solubilities of biologically active phenolic compounds: measurements and modeling, J. Phys. Chem. B, 113 (2009) 3469–3476.