RECOVERY OF VFAs FROM ANAEROBIC DIGESTION

OF DEPHENOLIZED OLIVE MILL WASTEWATERS BY ELECTRODIALYSIS

Alberto Scoma([#]), Felipe Varela Corredor, Lorenzo Bertin, Carlo Gostoli, Serena Bandini (*)

Department of Civil, Chemical, Environmental and Materials Engineering - Alma Mater Studiorum – University of Bologna – School of Engineering and Architecture.
 Via U. Terracini, 28, I-40131, Bologna, Italy

(*) corresponding author
e-mail : serena.bandini@unibo.it
tel +39 051 2090231
fax +39 0512090247

([#]) Present address: LabMET, University of Ghent, Coupure Links 653, 9000, Gent, Belgium

HIGHLIGHTS

- Removal of VFAs from actual site Olive Mill Wastewaters is feasible by Electrodialysis

- Membrane performances are not affected by processing Olive Mill Wastewaters.

- VFAs concentration factor ranges from 1.2 to 1.5.

- Acetate transport across the membrane increases after 50% NaCl removal

- Acetate enrichment in the concentrate is obtained as the main result of fractionation

ABSTRACT

Electrodialysis (ED) is applied for the first time as a separation-concentration step of the Volatile Fatty Acids (VFAs) enriched effluent resulting from fermentation of olive mill wastewaters. The process would represent a key step of an integrated multi-purpose biorefinery scheme including the biotechnological production of polyhydroxyalkanoates from acidified bioresidues.

A feasibility study is introduced by testing separation efficiency of membranes with synthetic model solutions of different compositions (sodium acetate and NaCl, or acetic-propionic-butyric acids with NaCl) and with actual OMW (a complex mixture of sodium salts of acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic, eptanoic acids, NaCl and other electrolytes).

Experiments were performed at room temperature in a lab-scale 2-compartments ED stack, containing Neosepta AMX-CMX membranes, at constant current density (31 A/m^2). Membrane resistance was tested by performing additional ED experiments according to a proper protocol developed in this work.

Removal of VFAs from actual OMW is feasible; no evidence of remarkable damages nor losses of membrane performance were observed after dedicated trials. Under the operative conditions investigated, VFAs removals ranged from 30 to 35%, resulting in a concentration factor between 1.2 and 1.5 with respect to the initial mother solution.

Chloride removal was remarkably high and it was greatly favoured at the beginning of operation; competition between chloride and acidic anions was remarkable as far as chloride concentration was high, whereas acidic anions transport across the membrane increased after NaCl removal overcame 50%.

Removal and concomitant concentration of VFAs were accomplished by a slight fractionation of acids with respect to the initial solution; the main outcome of which was an enrichment of acetate in the concentrate. Transport of each acidic anion across the membranes was affected by the concomitant role of concentration and diffusivity, which can shift the natural order imposed by the steric hindrance of the species.

1. INTRODUCTION

Organic acids are of primary importance in our society. They are largely used for the production of foods, beverages, pharmaceuticals, plastics, resins and other biochemical or chemical products [1-3]. Volatile Fatty Acids (VFAs) are linear short-chain aliphatic monofunctional compounds, which represent suitable precursors for the production of biopolymers, reduced chemicals (esters, ketones, aldehydes, alcohols and alkanes) and biofuels [4]. Large-scale production of VFAs is conventionally achieved by chemical synthesis, the most notable examples being acetic acid production by methanol carbonylation and propionic acid synthesis by the oxo process that involves reaction of ethylene and carbon monoxide to produce propionaldehyde, which is further catalytically oxidized. However, VFAs can be easily produced through the biotechnological exploitation of organic matrices by means of anaerobic digestion, as when operating under fermentative conditions [5-9]. Contrary to methanogenesis, energy conversion yields in fermentation are rather low and upper limits for overall VFAs concentration in acidic effluents are typically close to 20-30 g/dm^3 depending on the employed organic substrate, since inhibitory effects onto microbial consortia at low pH can interfere with proton gradient mechanisms [10]. Nonetheless, generation of VFA-enriched effluents is of pivotal importance to establish a carboxylate platform making use of highly impacting biowastes [11]. VFAs mixtures generated from renewable biowaste material could be used for the bioproduction of polyhydroxyalkanoates (PHAs) [37], which are microbial polyesters produced and stored by several bacteria [7]. The availability of VFA-enriched effluents would allow to feed PHA-producing processes without inducing significant dilution of the biomass.

Among several biowastes investigated as renewable resources, PHAs production from acidified olive mill wastewaters (OMWs) has been recently developed and described [12]. Economical sustainability of this process may benefit of an integration within a multi-purpose biorefinery scheme (Figure 1). OMWs can be pre-treated to recover polyphenols, which are natural antioxidants typically occurring in this biowaste, by means of a solid phase extraction process (SPE). Since OMW polyphenols can exert antimicrobial activities, SPE results into a dephenolized stream undergoing a more effective and performing fermentation of OMWs [8]. Application of an Electrodialysis (ED) separation process to the acidified effluent (OMW$_{acid}$) would generate a further VFA-enriched stream to be used for PHAs production in an aerobic fermentation step (AF). The

exhausted liquid (OMW$_{de-acid}$) produced by ED would thus represent an ideal aqueous effluent by which diluting solid biowastes in anaerobic digestion (AD) processes suitable for biomethane production. The present scheme (Fig. 1) represents only one of the many potential application which would benefit from the further refining of fermentation broths, as the availability (and feasibility) of sustainable recovery of VFAs and organics derived from biowaste management represents the main bottleneck for full development of second generation biorifineries [4,11].

The interest of membrane processes for separation and concentration of VFAs from fermentation broths is well documented in the literature [13-15]. Among membrane techniques, ED has been tested for various purposes [16-35] which were aimed at achieving different objectives related to the biotechnological production of VFAs, such as:

- Continuous removal of VFAs to increase the bioreactor productivity [17-24, 30,31]
- VFAs concentration [17,18,25-29]
- VFAs demineralization [26,26]
- Conversion of sodium salts of VFAs into the corresponding acids [18,19,35]
- Fractionation of the VFAs mixtures [12,13,29].

Feasibility of VFAs removal has been established and improvements of bioreactor productivity have been confirmed. That could be achieved by ED operating in different modes: high removals are documented by Jones et al. [20] by a combination of diffusion dialysis and conventional 2-compartments ED, by using a concentrate stream containing no VFAs. Demineralization is feasible as well, and it is highly efficient at very acidic conditions at which acids are not dissociated.

On the other hand, bipolar membranes were proposed to convert sodium acetate [32,33], sodium gluconate [34] and other salts [16] into the corresponding acids. Tests were performed above all with synthetic solutions containing single components. The use of bipolar membranes can overcome the typical drawbacks of conventional ED reducing salt pollution as well as the consumption of third components as proton source; however energy requirements are remarkably high and no data are available about application on actual fermentation broths.

Conversely, the concentration of VFAs by ED has not widely investigated yet: in 1999 Chukwu & Cheryan [17] successfully removed and concentrated acetic acid from glucose fermentation broths, despite other authors [18,19] had concluded in 1992 that ED was not a promising technique for the concentration of acetic and propionic mixtures owing to the presence of inorganic salts. The same authors, however, suggested ED as a good technique to maintain low acid levels in the fermentor, since removal was effective;   further, they obtained encouraging results in the fractionation of the

VFAs mixtures, which is rather difficult to perform owing to quite similar molecular dimensions and ion mobilities inside the membranes. Concerning VFAs fractionation, interesting reports about competitive transport of anions, such as formate, acetate, lactate and succinate have been recently supplied in the literature [35].

Generally speaking, ED has been widely applied for the recovery of small chain organic acids from aqueous solutions using synthetic solutions or fermentation broths, although most of investigations were conducted with a single selected compound. On the contrary, recovery of organic acids mixtures mainly derived from wastes has been poorly investigated in the literature [20,25].

Aim of this work is the assessment of the feasibility of a Conventional ED process for the treatment of the acidified olive mill wastewaters (OMW$_{acid}$) derived from the anaerobic acidogenic digestion (AAD) (see Fig.1). Primary objects are i) to test membrane resistance to complex actual agroindustrial effluents and ii) to check the feasibility of removal and concentration of VFAs together with indications about membrane performance and its reproducibility.

The possibility to obtain a concomitant fractionation of the VFAs-enriched effluent (see Fig.1) is also explored.

2. MATERIALS AND METHODS

2.1 Solutions

Experiments were performed using both actual VFA-rich effluents, which were obtained by fermenting a dephenolized OMW under anaerobic acidogenic conditions, and synthetic model VFA solutions.

Both the extraction of OMW polyphenols and the acidogenic fermentation of the dephenolized effluent were carried out according to procedures, which were previously designed, developed and assessed [7]. Briefly, polyphenols were recovered by a solid phase extraction procedure employing the commercial Amberlite XAD16 non-polar resin as the adsorbent, while the acidogenic bioprocess was carried out in a packed bed biofilm column reactor, which was filled with Vukopor S10 ceramic cubes (Lanik, CZ) as the packing material. Main features of the actual effluent (stream OMW$_{acid}$ of Fig.1) are reported in detail in Table 1. Importantly, almost 75% of the overall organic matter of the acidic effluent was represented by VFAs, as demonstrated by COD analyses. Before the ED operation, real solutions were clarified across 20 μm paper filter.

Model solutions were prepared by using reagent grade chemicals and de-ionized water in order to simulate the actual VFAs solutions, at different dilution levels; characteristics of model solutions are reported in Table 2.

2.2 Membranes and equipment

The apparatus used is a quite standard laboratory-scale two-compartments ED stack, manufactured by WORTMANN s.r.l. (Brescia, Italy) already tested in a previous study [36] and modified in our laboratory; details of the stack are reported in Table 3.

The cell pairs assembled in the stack are connected in parallel; 1 mm thickness PTFE spacers are used, whereas 1 mm thickness Viton gaskets are interposed between each membrane and the spacer, thus giving a 3 mm thickness chamber for fluids flow. Electrode chambers are assembled in PTFE also.

Homopolar membranes were tested; Neosepta CMX and Neosepta AMX were selected as cationic and anionic membranes, conditioned in Na-form and Cl-form, respectively.

Tests were performed in a lab-scale plant, operating in batch conditions; set-up is reported in Fig. 2. Three independent aqueous streams are involved: the diluate stream (compartment 1) and the concentrate stream (compartment 2) were continuously recirculated from the corresponding reservoirs to the ED stack; Na_2SO_4 solutions were used for electrodes rinse at acidic conditions (data are reported in detail in Tab.3). Tangential velocity through the spacers was close to 4.6 cm/s, both in the diluate and in the concentrate compartments.

Experiments were performed at constant electric current by measuring the increasing electric potential.

2.3 Experimental procedure

Experiments were performed at room temperature, starting with the same mother solution in the diluate and in the concentrate compartments in which pH values were adjusted in the range from 6.6 to 6.8 as initial conditions. That range was selected since it was quite close to the pH of actual OMW and high enough to guarantee the complete dissociation of all the organic acids present in the mixture. Constant electric current was kept at 500 mA (31 A/m^2); the electric potential was measured vs. time and experiments were stopped when the potential values reached 12 V. The current value is lower than the typical ones: in ref. [17] minimum values of 40 A/m^2 were measured, although in ref. [20] values of 4 A/m^2 were also considered.

Membranes were equilibrated for 30 minutes with test solutions by recirculating all the streams; after equilibration, the electric current was switched on and the ED process was assumed to be at its initial condition. No temperature control was maintained, temperature was measured in the range from 20 to 30 °C during experiments.

Three categories of ED experiments were carried out in duplicate by testing different solutions. Experiments with model solutions M1 and M2 (Table 2) and with actual OMW streams ("real OMW1" – Table 1) were performed. Data in Tables and Figures represent the average value of independent experiments made in duplicate, being the mean error lower than 5%..

A protocol was defined both for the start-up of a new set of virgin membranes and for the washing procedure; suggestions from the membrane technical bulletin were partially accounted for.

After each experiment with model solutions as well as with "real OMW1", a washing procedure was carried out before repeating experiments or for membrane storage. As a whole, the assessed protocol entailed the following steps:

a) membrane equilibration in the stack with test solution, no electric current
b) "base test"
c) rinsing with demi-water
d) membrane equilibration in the stack with test solution, no electric current
e) trial with model solutions or real OMW mixtures
f) rinsing with demi-water
g) membrane equilibration in the stack with test solution, no electric current
h) "base test"
i) washing procedure

Membranes were stored in 30 g/dm^3 NaCl-water solution; the same solution was used for membrane conditioning inside the stack and to preserve membranes inside the stack during the periods of inactivity.

The washing procedure was performed in batch conditions, according to the following steps. A basic cleaning step (aqueous solution of 10 g/dm^3 NaOH + 20 g/dm^3 NaCl at 150 dm^3/h and room temperature, 30 minutes long) was alternated with an acidic cleaning step (aqueous solution of 10 g/dm^3 HCl at 150 dm^3/h and room temperature, 30 minutes long). Each cleaning step was followed by 3-4 rinse steps with de-ionized water (10-15 microS/cm) performed at 150 dm^3/h and room temperature; rinsing is concluded when pH and conductivity values in the diluate and in the concentrate tank correspond to those observed for de-ionized water.

The final step of the washing procedure was "membrane conditioning" with aqueous solution of 30 g/dm^3 NaCl at 150 dm^3/h and room temperature, 30 minutes long.

Washing procedure was aimed to perform experiments with membranes at their best conditions, to avoid that fouling might hinder the real membrane performances.

Five cm^3 samples were taken from diluate and concentrate tanks at regular time intervals of 15 minutes during the course of each experiment: conductivity, pH, chloride and acids concentrations were measured; total volumes of each tank were measured also.

2.3.1 Base tests

In addition to the experiments with model and actual solutions, the so-called "base tests" were scheduled with the specific aim to test if any variation occurred in membrane performances after the operation with solutions containing VFAs. Such a test was essentially represented by a further ED experimental trial performed at room temperature, at constant electric current of 31 A/m^2, with 3 g/dm^3 NaCl water solutions at pH equal to 6.5 both in the diluate and in the concentrate compartments, at the same operative conditions reported in Table 3. After each trial, a "base test" was performed and the results were compared with those obtained in the corresponding "base test" performed before the experiment under investigation. Comparisons among all the "base tests", including the first "base test" with virgin membranes, are useful to check on variation in membrane performances.

"Base tests", were also subjected to sampling (5 cm^3) from diluate and concentrate tanks every 15 minutes: the measured quantities were the electric potential vs. time and conductivity in the tanks vs. time; NaCl concentrations in the diluate and in the concentrate compartments were obtained straightforwardly by simply using a conductivity meter.

2.3.2 Analytical procedures

VFAs concentration was monitored through a gas-chromatograph (HP GC-5890, Agilent, Milano, Italy) equipped with a FID detector and a Supelcowax-10 column (Sigma-Aldrich, Milano, Italy) under the following conditions: initial temperature 60°C; isothermal for 1 min; temperature rate 25°C/min; final temperature 150°C; isothermal for 6 min; temperature rate 4°C/min; final temperature 180°C; temperature rate 25°C/min; final temperature 240°C; injector and FID temperature 280°C; carrier gas flow rate (nitrogen) 17.6 mL/min. Before the analyses, samples were diluted with an equal amount of a 60 mM oxalic acid solution.

Inorganic anions concentration was measured by using a Dionex DX-120 IC system equipped with an IonPac AS14 4 × 250 mm column and a conductivity detector in combination with an ASRS-Ultra conductivity suppressor system (Dionex Corp.).

Unless otherwise mentioned, the concentration of anions (acetate, propionate, butyrate, etc.) are reported in terms of the corresponding acid (acetic acid, propionic acid, butyric acid, etc.).

3. PARAMETERS AND DATA PRESENTATION

Experimental data vs. time were elaborated according to the following procedure; some relevant parameters were defined and used to document the process efficiency and to compare membrane performances.

For the generic component j (organic acid and/or chloride), the following quantities are considered:

a) molar cumulative transport into concentrate compartment, evaluated at the generic time t:

$$N_j(t) = \frac{n_j^{(2)}(t) - n_j^{(2)}(0)}{t} \tag{1}$$

b) cumulative removal from the diluate into the concentrate compartment, evaluated at the generic time t:

$$rem_j(t) = \frac{n_j^{(2)}(t) - n_j^{(2)}(0)}{n_j^{(1)}(0)} \tag{2}$$

in which $n(0)$ represents the amount of substance at the initial condition in the corresponding compartment.

The interest for these two quantities is related to the need of quantifying the net transport from the diluate into the concentrate compartment, with the aim to exclude by calculation possible leakages towards the electrode rinse solutions.

Cumulative removal is used also to quantify performances of total VFAs.

For a monovalent anionic specie j, as it is the case under investigation, the transfer number can be accounted:

c) transfer number

$$T_j = \frac{N_j\, F}{n_c\, I} \tag{3}$$

Process efficiency and membrane performances vs. time can be described by the following quantities:

d) mass ratio of organic acid **j** in the diluate (1) (or in the concentrate (2)) compartment

$$\left.\frac{acid}{VFAs}\right|_{(1)} = \left.\frac{c_j(t)}{c_{VFAs,tot}(t)}\right|_{(1)} \tag{4}$$

e) enrichment factor of acid in the concentrate compartment

$$EF = \frac{\left.\dfrac{c_{acid}(t)}{c_{VFAs,tot}(t)}\right|_{(2)}}{\left.\dfrac{c_{acid}(0)}{c_{VFAs,tot}(0)}\right|_{(2)}} \tag{5}$$

f) separation ratio of acid (A) with respect to acid (B)

$$\alpha_{AB} = \left.\frac{EF_A}{EF_B}\right|_{(2)} \tag{6}$$

in which c is the mass concentration of the specie.

Of course, the interest for the definitions (4) to (6) is focussed to quantify the acids fractionation.

Finally, current efficiency and the energy consumption can be obtained by the following parameters:

g) total current efficiency

$$\varepsilon_c = \frac{\sum_j N_j\, F}{n_c\, I} = \sum_j T_j \tag{7}$$

h) overall specific energy requirement (total electric power per mole of total VFAs transported)

$$W = \frac{\int_0^{t_f} I \times V(t) dt}{N_{VFAs}(t_f) \times t_f}$$
(8)

in which W can be defined with respect to each "base test" also, that is referring to the unit mole of NaCl transported.

4. RESULTS AND DISCUSSION

4.1 Base tests

The results obtained in the "base tests" are reported in Figure 3 (experimental conditions are listed in Table 3).

The first trial performed is marked as "virgin-zero current", which corresponds to a base test carried out without electric current with the aim of checking the stack sealing: results indicated that the stack was well assembled and no leakages occurred across the spacers nor the gaskets.

The second chronological trial, defined as "virgin", was the first one performed in the presence of electric current; that is the basic reference with which the subsequent tests should be compared to check the membrane resistance.

Virgin membranes performances were quite typical, thus indicating a good overall operation of the system: an increase of conductivity can be observed in the concentrate compartment, corresponding to an increase in the salt concentration, in concomitance with a decrease of conductivity in the diluate compartment (Fig.3a)).

Base tests performed after ED trials with both synthetic and actual site OMWs show that membranes performances were not generally affected by the presence of organics. In addition, NaCl molar cumulative transport shows a good reproducibility with nearly constant values as a function of time, as it should be expected in a constant current density operation mode of the process (Fig.3b)). During all the base tests, neither sensible volume variations nor pH changes were measured in the two compartments; current efficiency was calculated close to 90-94 %.

4.2 Model solutions

12

The results obtained in the experiments with "model solutions" are reported in Figures 4 to 7 (characteristics of model solutions are reported in Table 2, experimental conditions are listed in Table 3).

During the experiments with M1 and M2 solutions, no sensible volume changes were observed in the two compartments, whereas a slight variation in the pH values was detected, as reported in the captions.

Model solution M1, containing only sodium acetate and sodium chloride, was tested in order to check the general trend of the process; conductivity data reported in Fig.4 are representative of a correct and good behaviour of the process. As previously noted [17-19], chloride and acetate ions pass across the membranes: in this case, the chloride and acetate removals (Fig.5d)) are accomplished by a concentration ratio in the compartment (2) of 2.02 and of 1.53, respectively (Fig.5a)), with a total current efficiency very close to 90% (Fig.5c)). The capability of the process to remove and concentrate acetic acid is rather evident.

Calculated transfer numbers of each specie (Fig5c)) put in evidence a strong competition between chloride and acetate: as far as the chloride concentration in the diluate is high, chloride is preferentially transported by the electric current; only after the chloride removal overcomes 50%, the acetate transport becomes to be favoured by far.

Model solution M2, containing NaCl and a mixture of VFAs (acetic, propionic, butyric), was tested as a rough simulation of agroindusrial fermented effluents. Once again, conductivity data reported in Fig.4 are representative of a correct and good behaviour of the process. In Fig. 6a) an increase of the total VFA concentration is observed in the compartment (2) vs. time, which clearly shows that the process is efficient both in the VFAs removal and in their concentration. In the final conditions (Fig. 7a)), total VFA removal approached 35%. Conversely, chloride removal is reconfirmed as a very high value (greater than 90%).

With regards to the transport of each single anions, in Fig. 6b) and 6c) the mass ratios as defined in Eq.(4) are reported vs. time. Although each acidic specie is transported towards the concentrate compartment, the membrane separation efficiency was different for each of them. The VFAs concentration is really accomplished by a slight fractionation: the final values of the enrichment factor (reported in Fig. 7b) put in evidence that the concentrate compartment is richer in acetate ions than the initial mother solution, whereas it is poorer in all the other acids. Although the extent of fractionation is not high, separation between acetic acid and butyric acid is possible to a certain

degree (separation ratio of 1.14 was achieved) , being that the separation between acetic acid and propionic acid is possible to lower extent (separation ratio of 1.10 was observed) .

The results here obtained, on the role played by ions competition and above all by the salts transport, are in partial agreement with other authors [18,19,35]. In this respect, the minor extent of separation [18] allows for process improvement.

4.3 Actual solutions

The results obtained in the experiments with "real solutions" are reported in Figures 4, and in Figures 8 to 10 (characteristics of real OMW1 solutions are reported in Table 1, experimental conditions are listed in Table 3). Actual site fermented OMW solution was tested in duplicate. Use of OMW did not result into sensible volume changes between the two compartments, whereas a slight variation in the pH values was detected, as reported in the captions of the corresponding figures.

Conductivity data (Fig.4) are representative of a correct and good behaviour of the process and, notably, the profile observed for model and real solutions was similar. The main differences can be related to different concentration values and to a more complex organic matrix still containing residual amounts of polyphenols and other undetected soluble organics, which taken together are responsible for about 25% of the overall OMW_{acid} organic matter (Table 1). In Fig. 8 an increase of the total VFAs concentration in the compartment (2) vs. time indicates that the process is efficient both in the VFAs removal and in their concentration. In the final conditions (Fig. 10a)), total VFAs removal approached 30% and the concentration ratio in the compartment (2) was obtained as 1.31, correspondingly; chloride removal is reconfirmed as a very high value (greater than 95%). The competition between chloride and the VFA anions is self-evident also (Fig.8). After the start-up of the process, in which chloride is preferentially transported across the membrane, acetate ion becomes the more easily transported VFA: after 60 minutes the acetate transfer number was almost equal to zero, whereas after 180 minutes increased up to 0.85. Since, at that time, conductivity of the diluate was still acceptable (Fig.4) and pH values were nearly constant and greater than the corresponding pKa of all the acids, prolongation of the process might yield better results. A quite similar trend was also observed in the case of model solution M2, although the effect was not so remarkable (Fig. 6a)).

As already noted with model solutions, concentration of VFAs from fermentation is accomplished by a slight fractionation (Fig.10b)): the final value of the enrichment factor approached unity for butyric acid, whereas it was measured close to 1.10 for acetic acid and 0.82 for the eptanoic acid. Notwithstanding experimentation requires further validation, basing on the results in Fig. 10 some conclusive remarks can be drawn to address future research on this topic. In Table 4, the final cumulative removal and the initial concentration of each acidic anion are reported in decreasing order; each of these is compared with the corresponding steric hindrance values which in turn are reported in increasing order. Since the ionic flux through the membranes, J_i, can be generally related to the electrochemical potential gradient of the ion itself existing across the membrane according to the typical relationship reported in Eq. (9), some observations are in order.

$$J_i \propto -D_i \nabla c_i - z_i c_i D_i \frac{F}{RT} \nabla V \qquad (9)$$

The scale of removals is the result of the contribution of ionic migration under the electric field and of concentration gradient. The former acts in the desired direction (from diluate to concentrate) and depends on concentration ad on steric hindrance, through the diffusivity value inside the membrane, whereas the latter acts in the opposite direction (from concentrate compartment to diluate compartment). The net flux of an anion across the membrane is then the result of those components which should be accurately taken into consideration and controlled in order to pursue the expected separation. The best example of this integrated response may be the one observed with caproic acid (Table 4), whose removal was better than what could be expected based solely on its steric hindrance.

Finally, the study can be completed by the quantification of energy consumption; in Fig. 11 the overall specific energy requirement is reported for model and actual site streams and it is compared with the corresponding "Base tests". Apparently, energy requirement in the case of real OMW1 solutions is lower than the values required for synthetic solutions, in view of the higher conductivity of OMW1 with respect to both model solutions M1 and M2 (see also Fig.4). The good membrane performances and their reproducibility is supported by the total cumulative % removal of NaCl which nearly approached the same value. However, by repeating the "Base Tests" after model solutions M2, membrane performances underwent a sensible variation of performances indicating a reduction of the electric resistance of the membrane. That behaviour was reconfirmed by the "base

test" after OMW1. Further experiments are needed to elucidate the reasons for this atypical behaviour which however is not suggestive of membrane deterioration. In fact, no sensible variations were observed in the molar cumulative transfer of NaCl vs. time (see Fig. 3) and a visual inspection after stack opening did not reveal any deterioration or damage.

5. CONCLUSIONS

To the very best of our knowledge, the present study reports on the first use of Conventional Electrodialysis on pre-treated Olive Mill Wastewaters.

Basically, applicability of Conventional Electrodialysis has been established for the recovery of Volatile Fatty Acids (VFAs) coming from anaerobic acidogenic digestion of Olive Mill Wastewaters. Both model and actual solutions were tested with the manifold aims of testing membrane resistance, quantifying VFAs removal and the corresponding concentration factor, as well as testing separation efficiency of the removed acids. Removal of VFAs from actual OMW solutions is feasible at room temperature and pH values close to 6-6.5 with no damage of membranes; membrane performances were not affected by the contact with real solutions. Under the operative conditions investigated, VFAs removals were about 30-35% and resulted into a concentration factor between 1.2 and 1.5 with respect to the initial solution.

The process was accompanied by a remarkably high chloride removal which was greatly favoured at the beginning of operation. Competition between chloride and acidic anions occurred as long as chloride concentration was high, whereas acidic anions transport across the membrane increased after NaCl removal overcame 50%. This conclusion is in agreement with previous papers which rejected Elecrodialysis as a non-suitable process. Although not impacting further bioprocessing of non-saline bioresidues such as the present OMWs, this observation should be taken into account to further address the selection of best operative conditions in order to inhibit or limit chloride transport across the membrane. Nonetheless, because of the nature of this process, application to saline bioresidues such as molasses [11] would probably be unprofitable.

The removal and the contemporary concentration of VFAs were accomplished by a slight fractionation of acids with respect to the initial mother solution; acetate enrichment in the concentrate was obtained as the main result. Transport of each acid anion across the membranes was affected by the concomitant role of concentration and diffusivity, which can shift the natural

order imposed by the steric hindrance of each species. That conclusion gives suggestions and indications about which actions can be pursued in order to enhance the flux of some organics and to inhibit the flux of other compounds, at the same time.

The present paper indicates that the use of ED for VFAs recovery is feasible, although its application on industrial scale needs to address a number of issues. The process is highly affected by low conductivity in the diluate compartment; however, increase in the separation efficiency may be achieved by longer ED operation. The role of pH should be accurately investigated since the separation efficiency can be highly affected by the relative dissociation of each acidic organic species.

NOTATIONS

abbreviations

ace   acetic acid

pro   propionic acid

ibut   isobutyric acid

but   butyric acid

ival   isovaleric acid

val   valeric acid

icap   isocaproic acid

cap   caproic acid

ept   eptanoic acid

VFAs   Volatile Fatty Acids

symbols

0	initial time	
c	molar / mass concentration	mol m^{-3} / kg m^{-3}
n	amount of substance	mol
n_c	number of elementary cells	
rem	cumulative removal (defined in eq. (2))	
t	generic time	
EF	enrichment factor (defined in eq. (5))	
F	Faraday constant	C mol^{-1}
I	electric current	A
J	molar flux	mol m^{-2} s^{-1}
N	molar cumulative transport (defined in eq. (1))	mol s^{-1}
R	universal gas constant	
T	Thermodynamic temperature	K
T_j	transfer number (defined in eq. (3))	

V	electric potential	V
W	overall specific energy requirement(defined in eq. (8))	J mol^{-1}

α	separation ratio (defined in eq. (6))	
ε_c	current efficiency (defined in eq. (7))	

subscripts and superscripts

j	generic specie
(1)	diluate compartment
(2)	concentrate compartment

REFERENCES

[1] J.A. Dean, Lange's Handbook of Chemistry, McGraw-Hill Book Co., NewYork, 1985.

[2] H. Takahashi, K. Ohba, K.I. Kikuchi, Sorption of mono-carboxylic acids by an anion-exchange membrane, Biochem. Eng. J. 16 (2003) 311– 315.

[3] H. Takahashi, K. Ohba, K.I. Kikuchi, Sorption of di- and tri carboxylic acids by an anion-exchange membrane, J. Membr. Sci. 222 (2003) 103–111.

[4] M.T. Agler, B.A.Wrenn, S.H. Zinder, Angenent LT. Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. Trends Biotechnol, 29 (2011) 70-78.

[5] R.Khiewwijit, K.L.Keesman, H. Rijnaarts, H.Temmink, Volatile fatty acids production from sewage organic matter by combined bioflocculation and anaerobic fermentation, Bioresource Technology 193 (2013) 150-155

[6] F.Bonk, J.R. Bastidas-Oyanedel, J.E. Schmidt, Converting the organic fraction of solid waste from the city of Abu Dhabi to valuable products via dark fermentation – Economic and energy assessment, Waste Management 40 (2015) 82-91

[7] G. Braunegg, G. Lefebvre, K.F. Genser, Polyhydroxyalkanoates, biopolyesters from renewable resources: physiological and engineering aspects, Journal of Biotechnology, 65 (1998) 127-161.

[8] A. Scoma, L.Bertin, G. Zanaroli, S.Fraraccio, F.Fava,   A physicochemical–biotechnological approach for an integrated valorization of olive mill wastewater, Biores. Technol. 102 (2011) 10273-10279

[9] M. Beccari, L. Bertin, D. Dionisi, F. Fava, S. Lampis, M. Majone, F. Valentino, G. Vallini, M. Villano, Exploiting olive oil mill effluents as a renewable resource for production of

biodegradable polymers through a combined anaerobic-aerobic process, J. Chem. Technol. Biotechnol., 84 (2009) 901-908

[10] J. Yu, J. Wang, Metabolic flux modeling of detoxification of acetic acid by Ralstonia eutropha at slightly alkaline pH levels, Biotechnol. Bioeng. 73 (2001) 458-464.

[11] A.Scoma, S.Rebecchi, L.Bertin, F.Fava, High impact biowastes from South European agro-industries as feedstock for second generation biorefineries, Critical Reviews in Biotechnology, (in press), DOI: 10.3109/07388551.2014.947238

[12] G.A. Martinez, L. Bertin, A. Scoma, S. Rebecchi, G. Braunegg, F. Fava, Production of polyhydroxyalkanoates from dephenolised and fermented olive mill wastewaters by employing a pure culture of Cupriavidus necator. BIOCHEM. ENG. J. 97 (2015) 92-100

[13] M.G.Buonomenna, J.Bae, Membrane processes and renewable energies, Renewable and Sustainable Energy reviews, 43 (2015) 1343-1398

[14] Y.Zhang, I.Angelidaki, Bioelectrochemical recovery of waste-derived volatile fatty acids and production of hydrogen and alkali, Water Research 81 (2015) 188-195

[15] S.K.Choudhari, F.Cerrone, T.Woods, K. Joyce, V.O'Flaherty, K. O'Connor, R.Babu, Pervaporation separation of butyric acid from aqueous and anaerobic digestion (AD) solutions using PEBA based composite membranes, Journal of Industrial and Engineering Chemistry, 23 (2015) 163-170

[16] C. Huang, T. Xu, Y.Zhang, Y. Xue, G. Chen, Application of electrodialysis to the production of organic acids: state-of-the-art and recent developments, J. Membr. Sci., 288 (2007) 1-12

[17] U.N. Chukwu, M.Cheryan, Electrodialysis of acetate fermentation broths, Appl. Biochem. Biotechnol., 77-79 (1999) 485-499

[18] S.T. Zhang, H. Matsuoka, K. Toda, Production and recovery of propionic and acetic acids in electrodialysis culture of Propionibacterium shermanii, J. Ferment. Bioeng., 75 (1993) 276-282

[19] A.J. Weier, B.A. Glatz, C.E. Glatz, Recovery of propionic and acetic acids from fermentation broth by electrodialysis, Biotechnol. Prog., 8, (1992) 479-485

[20] R.J.Jones, J.Massanet-Nicolau, A.Guwy, G.C. Premier, R.M.Dinsdale, M.Reilly, Removal and recovery of inhibitory volatile fatty acids from mixed acid fermentations by conventional electrodialysis, Bioresource Technology 189 (2015) 279-284

[21] M. Wong, J.M. Woodley, G.J. Lye, Application of bipolar electrodialysis to *E.coli* fermentation for simultaneous acetate removal and pH control, Biotechnol Lett, 32 (2010) 1053-1057

[22] Y. Nomura, M. Iwahara, M. Hongo, Continuous production of acetic acid by electrodialysis bioprocess with a computerized control of fed batch culture, J. Biotechnol., 12 (1989) 317-326

[23] J.Tang, S. Jia, S.Qu, Y.Xiao, Y.Yan, N.Ren, An integrated biological hydrogen production process based on ethanol-type fermentation and bipolar membrane electrodyalisis, Int. J. of Hydrogen Energy, 39 (2014) 13375-13380

[24] B. Zelic, D. Vasic-Racki, Process development and modeling of pyruvate recovery from a model solution and fermentation broth, Desalination 174 (2005) 267–276.

[25] Z. Wang, Y. Luo, P. Yu, Recovery of organic acids from waste salt solutions derived from the manufacture of cyclohexanone by electrodialysis, J. Membr. Sci., 28 (2006) 134-137

[26] J.Xu, X.Su, J.Bao, Y.Chen, H.Zhang, L.Tang, K.Wang, J.Zhang, X.Chen, Z.Mao, Cleaner production of citric acid by recycling its extraction wastewater treated with anaerobic

digestion and electrodialysis in an integrated citric acid-methane production process, Bioresource Technology, 189 (2015) 186-194

[27] M. Fidaleo, M. Moresi, Assessment of the main engineering parameters controlling the electrodialytic recovery of sodium propionate from aqueous solutions, J. Food Eng. 76 (2006) 218–231.

[28] G.S. Luo, S. Pan, J.G. Liu, Use of the electrodialysis process to concentrate a formic acid solution, Desalination 150 (2002) 227–234.

[29] I.N. Widiasa, P.D. Sutrisna, I.G. Wenten, Performance of a novel electrodeionization technique during citric acid recovery, Sep. Purif. Technol. 39 (2004) 89–97.

[30] H. Takahashi, K. Ohba, K.I. Kikuchi, Sorption of mono-carboxylic acids by an anion-exchange membrane, Biochem. Eng. J. 16 (2003) 311– 315.

[31] H. Takahashi, K. Ohba, K.I. Kikuchi, Sorption of di- and tri carboxylic acids by an anion-exchange membrane, J. Membr. Sci. 222 (2003) 103–111.

[32] L. Yu, Q. Guo, J. Hao, W. Jiang, Recovery of acetic acid from dilute wastewater by means of bipolar membrane electrodialysis, Desalination, 129 (2000) 283-288

[33] L. Yu, T. Lin, Q. Guo, J. Hao, Relation between mass transfer and operation parameters in the electrodialysis recovery of acetic acid, Desalination, 154 (2003) 147-152

[34] S. Novalic, T. Kongbangkerd, K.D. Kulbe, Recovery of organic acids with high molecular weight using a combined electrodialytic process, J. Membr. Sci., 166 (2000) 99-104

[35] P.J. Moon, S.J. Parulekar, S.P. Tsai, Competitive anion transport in desalting of mixtures of organic acids by batch electrodialysis, J. Membrane. Sci., 141 (1998) 75-89

[36] S.Bandini, Nafion membranes for conversion of sodium phenoxides into undissociated phenols, J. Membrane. Sci., 207 (2002) 209-225.

[37] L.S. Serafim, P.C. Lemos, M.G.E. Albuquerque, M.A.M. Reis, Strategies for PHA production by mixed cultures and renewable waste materials, Appl Microbiol Biotechnol (2008) 81:615-628

Figures

Captions for Figures and Tables

Table 1. characteristics of real Olive Mill Wastewaters after anaerobic acidogenic digestion

Table 2 Characteristics of model solutions.

Table 3. Details of the ED stack and main operating conditions.

Table 4. Scale of cumulative removal and concentration at initial conditions in comparison with steric hindrance.

Fig.1. Schematic flow sheet of the proposed overall process. Effluents: olive mill wastewater (OMW), dephenolized olive mill wastewater (OMW$_{deph}$), acidified olive mill wastewater (OMW$_{acid}$), VFA-enriched effluent (VFAs), deacidified olive mill wastewater (OMW$_{deacid}$). Operations: solid phase extraction of polyphenols (SPE), VFAs production by anaerobic acidogenic digestion (AAD), VFAs concentration by electrodialysis (ED), PHAs production by aerobic fermentation (AF), biomethane production by anaerobic digestion (AD).

Fig. 2. Experimental set-up.

Fig.3. Base tests vs. time at 25 °C: comparison between performances at zero current and at 31 A/m^2 of virgin membranes and after VFAs trials..
a) conductivity
b) molar cumulative transfer of NaCl from diluate and into the concentrate at 31 A/m^2.
open symbols=concentrate; closed symbols=diluate.

Fig. 4. ED of VFAs at 25°C and 31 A/m^2: conductivity vs. time in trials with model solutions M1, M2 and real solution OMW1.
open symbols=concentrate; closed symbols=diluate.

Fig.5. ED of model solution M1: experimental results vs. time, at 25°C and 31 A/m^2.
a) acetic acid and chloride concentration; b) molar cumulative transfer of acetic acid and of chloride from diluate and into concentrate; c) transfer number of acetate and of chloride and total current efficiency; d) % cumulative removal of acetic acid and of chloride.
initial/final pH in (1) = 6.6/6.1; initial/final pH in (2) = 6.6/7.0;
(1)=diluate compartment; (2)=concentrate compartment

Fig.6. ED of model solution M2: experimental results vs. time, at 25°C and 31 A/m^2.
a) total VFAs and chloride concentration; b) mass ratio of acids and chloride % decrease in the diluate compartment c) mass ratio of acids and chloride % increase in the concentrate
initial/final pH in (1) = 6.8/6.6; initial/final pH in (2) = 6.8/7.0;
(1)=diluate compartment; (2)=concentrate compartment; (0) = initial value

Fig.7. ED of model solution M2 at 25°C and 31 A/m^2: final conditions.
a) % cumulative removal of acids and chloride; b) enrichment factor of acids into concentrate compartment.

Fig.8. ED of real solution OMW1: total VFAs and chloride concentration vs. time in the concentrate and in the diluate compartments, at 25°C and 31 A/m^2.
initial/final pH in (1) = 6.42/6.39; initial/final pH in (2) = 6.43/6.51;
(1)=diluate compartment; (2)=concentrate compartment

Fig.9. ED of real solution OMW1: experimental results vs. time, at 25°C and 31 A/m^2.
a),b) mass ratio of acids and chloride % decrease in the diluate compartment;

c),d) mass ratio of acids and chloride % increase in the concentrate compartment

Fig.10 ED of real solution OMW1 at 25°C and 31 A/m^2: final conditions.
a) % cumulative removal of acids and of chloride; b) enrichment factor of acids into concentrate compartment.

Fig. 11. Overall specific energy requirement. Comparison between trials with VFAs (open symbols) and the corresponding Base Tests after use (closed symbols). Test 0 = Base Test with virgin membranes. Cumulative % removal of NaCl is also reported for each Base Test.

Table 1: characteristics of real Olive Mill Wastewaters after anaerobic acidogenic digestion

Total COD mg/ dm^3	VFA COD mg/ dm^3	VFA mg/ dm^3	Phenols mg/ dm^3	pH	Chloride mg/ dm^3	Nitrite mg/ dm^3	Nitrate mg/ dm^3	Sulphate mg/ dm^3
26200	19000	14500	120	6.22	514	39.1	absent	31.5

mg / dm^3								
acetic	propionic	isobutyric	butyric	isovaleric	valeric	isocaproic	caproic	eptanoic
7084.3	656.3	1157.0	2938.5	390.3	620.0	472.4	1562.4	538.0

Table 2: Characteristics of model solutions

Model solution	M1	M2
pH	6.6	6.8
conductivity at 25 °C (mS/cm)	5.2	7.8
NaCl (mg/dm^3)	870	870
Total VFAs (mg/dm^3)	3900	5820
acetic acid (mg/dm^3)	3900	2460
propionic acid (mg/dm^3)	------	900
butyric acid (mg/dm^3)	------	2460

Table 3: Details of the 2-compartment ED stack and main operating conditions

Cathode	AISI 316 SS
Anode	coated (Ru-Ir) Ti
Useful electrode area (m^2)	0.016
Number of elementary cells	3
Number of membranes (CMX + AMX)	4 + 3
Total membrane area (m^2)	0.112
Flow rate in diluate / concentrate (dm^3/h)	150 / 150
Initial volume in diluate/concentrate (dm^3)	4 / 4
Electrodes rinse solution (g/ dm^3)	Na$_2$SO$_4$ (130)
Rinse solution pH	3.5
Electrodes rinse initial volume (dm^3)	5
Flow rate in rinse solution (dm^3/h)	200

Table 4. Scale of cumulative removal and concentration at initial conditions in comparison with steric hindrance.

	removal	concentration	hindrance	
highest	ace	ace	ace	lowest
	but	but	pro	
	pro	ibut	ibut	
	ibut	cap	but	
	val	pro	ival	
	cap	val	val	
	ival	ival	icap	
	icap	icap	cap	
lowest	ept	ept	ept	highest
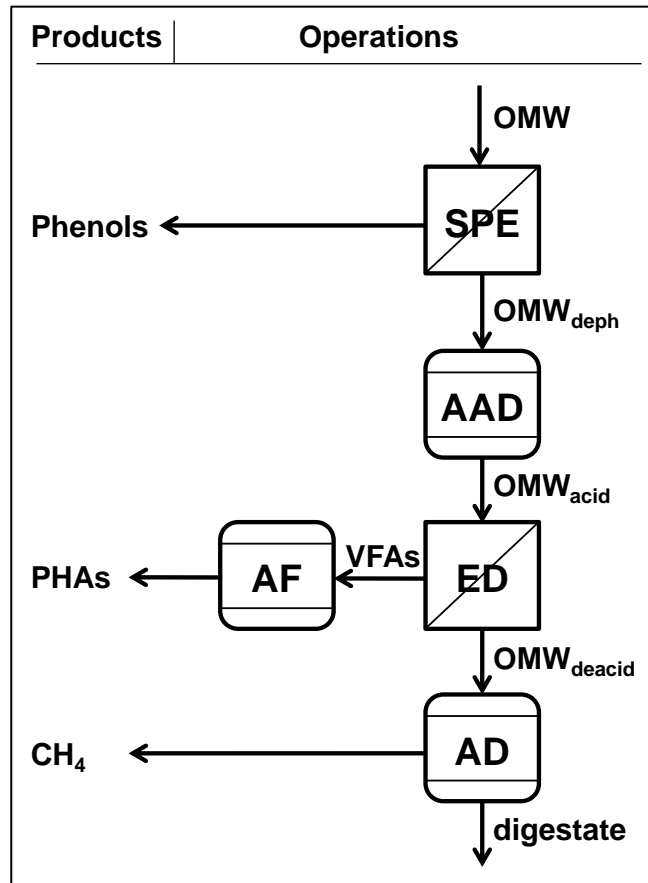
Fig.1. Schematic flow sheet of the proposed overall process. Effluents: olive mill wastewater (OMW), dephenolized olive mill wastewater (OMW$_{deph}$), acidified olive mill wastewater (OMW$_{acid}$), VFA-enriched effluent (VFAs), deacidified olive mill wastewater (OMW$_{deacid}$). Operations: solid phase extraction of polyphenols (SPE), VFAs production by anaerobic acidogenic digestion (AAD), VFAs concentration by electrodialysis (ED), PHAs production by aerobic fermentation (AF), biomethane production by anaerobic digestion (AD).
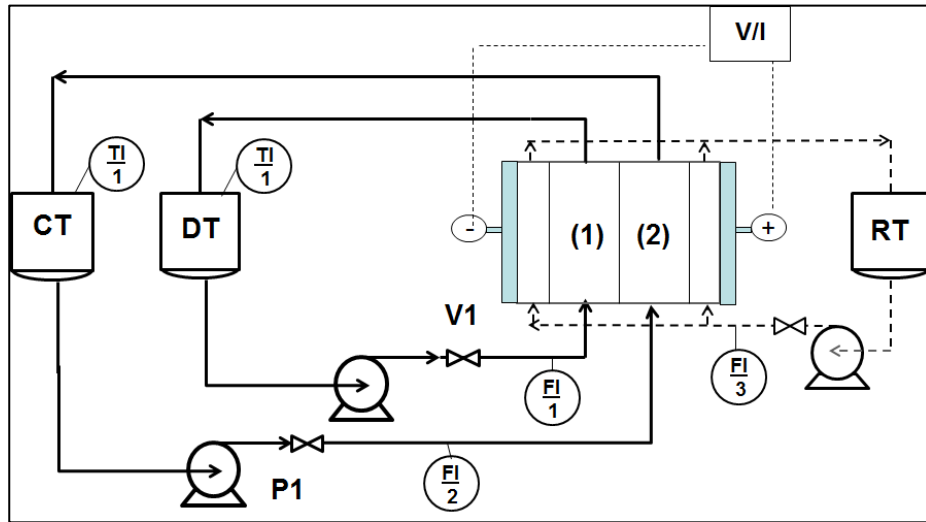
Equipment list		Instruments	
CT/ DT	*Concentrate / Diluate Tank*	*V1*	*Regulating valves*
RT	*Rinse solution Tank*	*TI*	*Thermometer*
P1	*Centrifugal Pump*	*FI*	*Flow meter*
V/I	*Power DC supply*		

Fig. 2. Experimental set-up.

Fig.3. Base tests vs. time at 25 °C: comparison between performances at zero current and at 31 A/m^2 of virgin membranes and after VFAs trials.
a) conductivity
b) molar cumulative transfer of NaCl from diluate and into the concentrate at 31 A/m^2.
open symbols=concentrate; closed symbols=diluate.

Fig. 4. ED of VFAs at 25°C and 31 A/m^2: conductivity vs. time in trials with model solutions M1, M2 and real solution OMW1. open symbols=concentrate; closed symbols=diluate.

Fig.5. ED of model solution M1: experimental results vs. time, at 25°C and 31 A/m^2.
a) acetic acid and chloride concentration; b) molar cumulative transfer of acetic acid and of chloride from diluate and into concentrate; c) transfer number of acetate and of chloride and total current efficiency; d) % cumulative removal of acetic acid and of chloride.
initial/final pH in (1) = 6.6/6.1; initial/final pH in (2) = 6.6/7.0; (1)=diluate compartment; (2)=concentrate compartment.

Fig.6. ED of model solution M2: experimental results vs. time, at 25°C and 31 A/m^2.
a) total VFAs and chloride concentration; b) mass ratio of acids and chloride % decrease in the diluate compartment c) mass ratio of acids and chloride % increase in the concentrate
initial/final pH in (1) = 6.8/6.6; initial/final pH in (2) = 6.8/7.0; (1)=diluate compartment; (2)=concentrate compartment; (0) = initial value

Fig.7. ED of model solution M2 at 25°C and 31 A/m^2: final conditions.
a) % cumulative removal of acids and chloride; b) enrichment factor of acids into concentrate compartment.

Fig.8. ED of real solution OMW1: total VFAs and chloride concentration vs. time in the concentrate and in the diluate compartments, at 25°C and 31 A/m^2.
initial/final pH in (1) = 6.42/6.39; initial/final pH in (2) = 6.43/6.51; (1)=diluate compartment; (2)=concentrate compartment
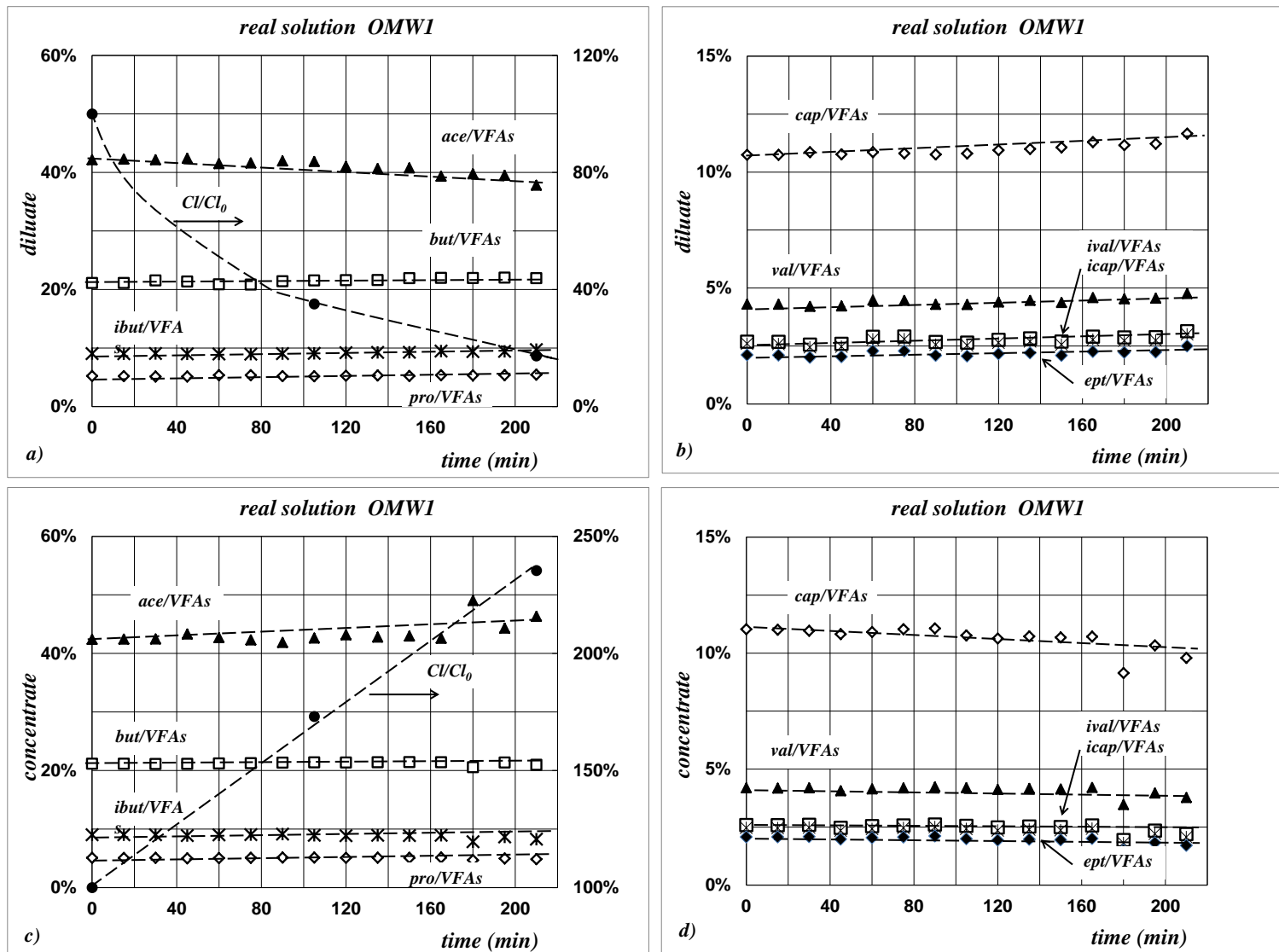
Fig.9. ED of real solution OMW1: experimental results vs. time, at 25°C and 31 A/m^2.
a),b) mass ratio of acids and chloride % decrease in the diluate compartment;
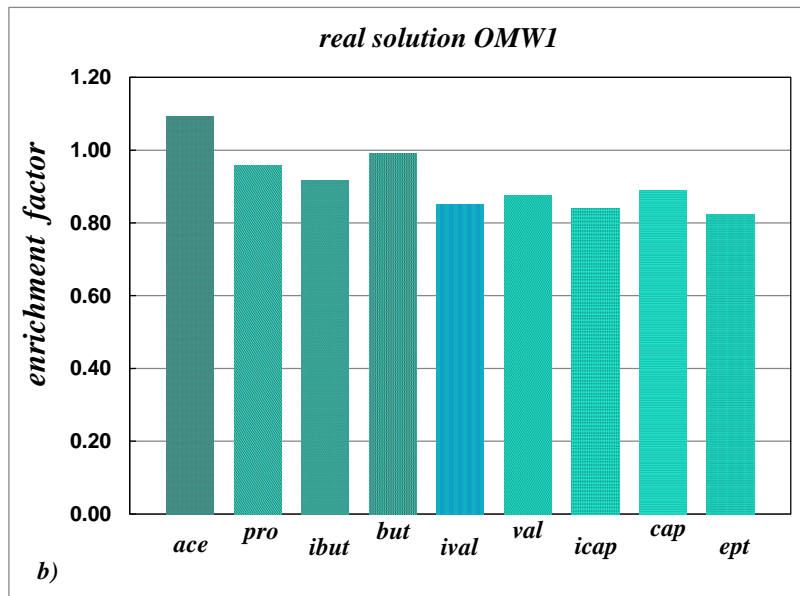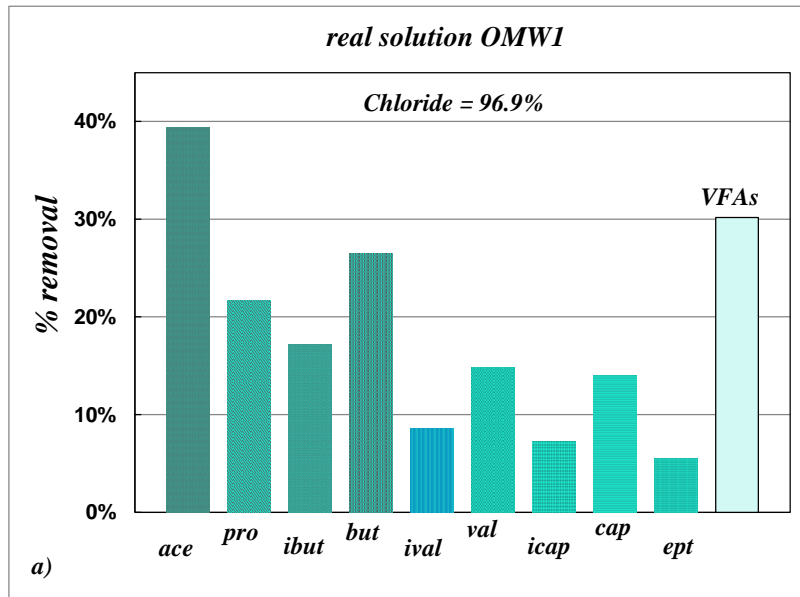c),d) mass ratio of acids and chloride % increase in the concentrate compartment

Fig.10 ED of real solution OMW1 at 25°C and 31 A/m^2: final conditions.
a) % cumulative removal of acids and of chloride; b) enrichment factor of acids into concentrate compartment.
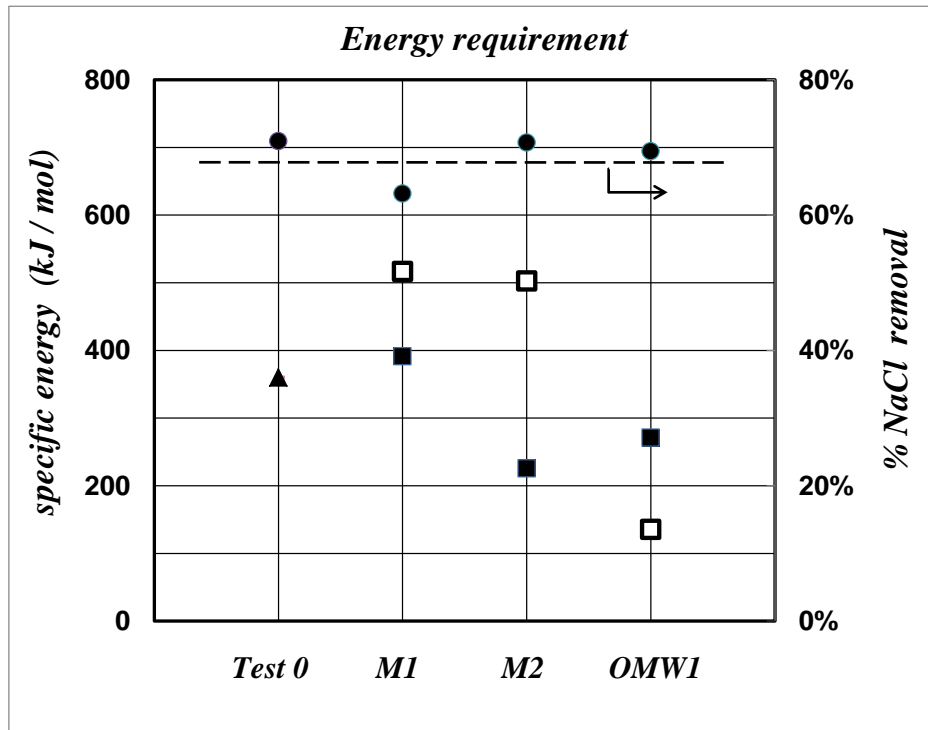
Fig. 11. Overall specific energy requirement. Comparison between trials with VFAs (open symbols) and the corresponding Base Tests after use (closed symbols). Test 0 = Base Test with virgin membranes. Cumulative % removal of NaCl is also reported for each Base Test.