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# Removal of inhibitory compounds from olive stone auto-hydrolysis liquors by nanofiltration

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**ABSTRACT**

This work aims to study the use of nanofiltration for the removal of metabolic inhibitory compounds, such as furfural and acetic acid, from olive stones auto-hydrolysis liquors. The performance of NF90 and NF270 nanofiltration membranes was first assessed in terms of permeability and rejection to target compounds in a total recycle mode of operation. Both auto-hydrolysis liquors and model solutions, containing xylose, glucose and furfural, were processed at pressures ranging from 4 to 20 bar. Significantly lower membrane permeability was observed in the processing of the auto-hydrolysis liquors, which could be associated to membrane fouling. Solute rejection results for liquors and model solutions were similar, with an almost total rejection of hexoses and pentoses, while furfural and acetic acid were allowed to permeate to a certain extent. In order to accomplish an effective removal of furfural and acetic acid, the auto-hydrolysis liquors were processed in a diafiltration mode of operation, maintaining a constant volume. In this way, the concentration of hexoses and pentoses in the liquor was kept constant, while furfural and acetic acid were depleted to the desired values. A mathematical model based on a mass-balance of the system was validated with experimental data, which enables its use for process optimization and scale-up.

*Keywords:* Membrane processing; Nanofiltration; Diafiltration; Removal of metabolic inhibitors; Olive stone; Autohydrolysis

## 1. Introduction

Olive stones are one of the lignocellulosic residues of the olive oil production and are readily available in the Mediterranean basin. Currently, their main application is as solid fuel [1]. However, its significant polysaccharide content makes this material a potential source of sugars that can find direct use or be converted to added-value products. For that to happen, olive stones must be previously pre-treated to release part of the hemicellulosic sugars and to facilitate the subsequent enzymatic saccharification of cellulose.

Hydrothermal processes are among the most promising pre-treatments [2–6]. One of the main advantages identified in such procedures is the production of oligosaccharides that can have significant market value, namely in the pharmaceutical, nutraceutical, and food industries [7,8] mainly due, but not restricted, to their

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prebiotic effects, or in biotechnology as growth substrate for the production of probiotics [9–11]. Nevertheless, potential metabolic inhibitory compounds such as aliphatic acids as acetic and formic acids, furfural, and monomeric phenolics can also be produced during the auto-hydrolysis and may hinder oligosaccharide recovery/purification or utilization.

Detoxification processes such as overliming, activated charcoal, solvent extraction, evaporation, or the use of ionic resins are usually applied, with relative success, to the detoxification of hemicellulosic hydrolysates. The main drawbacks are their poor selectivity and sugar loss due to unspecific adsorptions (e.g., activated charcoal [12]), that can be even more significant for oligomeric sugars [13]. Furthermore, oligosaccharides can also undergo thermal degradation, e.g., under harsher evaporation conditions. An alternative is the use of filtration-based processes where, although several works have already been carried out [7,14–16], the potential of membrane filtration for the effective fractionation of such compounds has not been fully explored. Recent work by Weng et al. [17,18] shows that nanofiltration has potential to be applied in the separation of sugars from furans and carboxylic acids. Actually, as the inhibitory compounds are characterized by molecular weights smaller/around 100 Da, and the sugars of interest have higher values, at least above 150 Da, the use of nanofiltration could be considered a good candidate for an efficient removal of such inhibitory compounds, with the further advantage of recovering the inhibitory compounds leading to their possible re-use or valorization as in the case of acetic acid, or phenolic compounds that may have relevant market value.

The aim of this work is to evaluate the use of nanofiltration in a diafiltration mode for the removal of furfural and acetic acid from liquors obtained from olive stones auto-hydrolysis.

## 2. Materials and methods

### 2.1. Materials

Olive stones were supplied by UCASUL – União de Cooperativas UCRL (Alvito, Portugal) and were sieve-separated from 2-phase olive pomace before industrial processing. Upon arrival the raw material was homogenized in a defined lot, and stored in plastic containers at room temperature. It contains, on a dry-basis: glucan, 29.41%; xylan, 29.19%; acetic acid, 3.20%; Klason lignin, 34.2%; ash, 0.88%; protein, 1.93%; fat, 0.94%; and others, 0.3% (by difference).

Glucose (99.5%) and xylose (<99%) and furfural (≥99%) used in model solutions were obtained from Sigma (Germany) and Fluka (Switzerland), while sodium hydroxide (pellets Gr p.a.) was obtained from Merck, (Germany). All standards and solvent ($H_2SO_4$) used for HPLC analysis were from analytical grade.

### 2.2. Autohydrolysis of olive stones

The hydrothermal treatments (auto-hydrolysis) were performed in a stainless steel reactor (Parr Instruments Company, Moline, Illinois, USA) with a total volume of 2 l. Olive stones were mixed with water in the reactor in order to obtain a liquid-to-solid ratio of 3 and 4 (kg water/kg dry raw material), and will be referred as liquors 1 and 2 throughout this work. The agitation speed was set at 150 rpm and the treatment was carried out under non-isothermal operating conditions to reach final temperatures of 200°C and 215°C for liquors 1 and 2, respectively. When the desired temperature was attained, the reactor was rapidly cooled down to room temperature by circulating water through a serpentine coil together with an ice bath.

### 2.3. Hydrolysate processing

The liquid and solid phases were separated in a hydraulic press (Sotel, Portugal) using a pressure up to 250 kg/cm$^2$. The liquid phase (liquor) was filtrated (Whatman filter paper no. 41) and stored at 4°C. If further required the liquors were centrifuged at 12,000 rpm for 12 min at 20°C (Hermle Z 323 K centrifuge, Germany) before processing, in order to remove suspended solids and prevent possible upstream problems, such as clogging, in the nanofiltration step.

### 2.4. Nanofiltration

The nanofiltration experimental setup, shown in Fig. 1, was comprised by a GE Sepa CF cross-flow module (GE Osmonics, USA), a high-pressure feed pump and a second pump for feeding water to the feed recipient controlled by a level indicator placed in the feed recipient. The module has an effective membrane area of 140 cm$^2$. Two commercial polymeric nanofiltration membranes, NF90 and NF270 (Dow USA), were selected for this

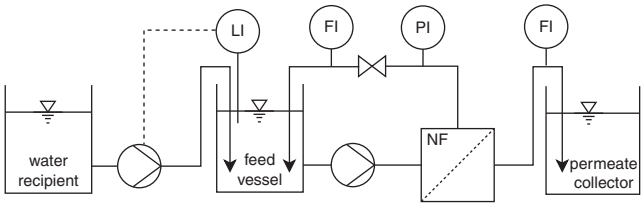


Fig. 1. Experimental nanofiltration setup. PI, LI and FI are respectively pressure, level and flow-rate indicators.

study. These membranes are characterized by molecular weight cut-offs (MWCO) of 150 and 400 Da, respectively. All nanofiltration experiments were conducted at 30°C and using a linear velocity of 0.4 m/s within the spacer-filled feed compartment, aiming at minimizing possible concentration polarization effects. Before use, new membrane pieces were subjected to compaction at 20 bar during 1 h until a constant permeability value was obtained. The membrane permeability was monitored after every experiment with deionized water. Alkaline cleaning with a 0.1% NaOH solution at 40°C and 1 bar was carried out when necessary to restore the membrane permeability to its initial value.

The membranes were firstly characterized in term of hydraulic permeability. Then, model feed solutions (GXF solutions) of 2 l containing Glucose (50 mm), Xylose (50 mm) and Furfural (10 mm) were prepared and processed by both membranes in a pressure range of 4–20 bar, under a total recycle mode of operation, in which both the feed and permeate streams were recirculated to the feed recipient. Autohydrolysis liquor 1 and 2 were also processed under the same operating conditions. Permeability and rejection values were determined in every experiment using the following relations:

$$J_V = L_p \left( \Delta P - \Delta \pi \right) \quad (1)$$

$$R_i = 1 - \frac{C_{i,p}}{C_{i,f}} \quad (2)$$

where $J_V$ is the solvent volumetric flux, $L_p$ is the membrane permeability, $\Delta P$ is the transmembrane pressure, $\Delta\pi$ is the osmotic pressure difference (determined by the van't Hoff equation), $R_i$ is the apparent rejection of solute $i$, and $C_{i,p}$ and $C_{i,f}$ are the concentration in the permeate and feed of solute $i$.

### 2.5. Analytical methods

Glucose, xylose, arabinose, acetic acid, 5-hydroxymethyl furfural (HMF) and furfural were analyzed by HPLC. The D-7000 HSM software was used associated to a Merck Hitachi LaChrome equipment with a L7000 interface module, a L7200 auto sampler, a L7490 RI detector, a L7350 column oven and a L7100 pump. An Aminex HPX-87H (7.8 × 300 mm) cation exchange column, from Bio-Rad (USA), was used at 65°C with a flow rate of 0.5 ml/min of 0.01 N $H_2SO_4$ solution used as the mobile phase. Oligosaccharides concentration was calculated from the increase in sugar monomers, as analysed by HPLC, after liquor acid post-hydrolysis. The samples and eluent were pre-filtered with 0.45 μm pore size filters from Pall. Conductivity and pH was measured with a Consort C861 multi parameter analyzer. Biomass composition was determined based on NREL Laboratory Analytical Procedures [19]. Protein quantification was performed by the Kjeldahl method using the $N \times 6.25$ conversion factor.

## 3. Results

### 3.1. Composition of the tested auto-hydrolysis liquors

During auto-hydrolysis, the more labile hemicellulosic polysaccharides present in the lignocellulosic biomass are solubilyzed and hydrolyzed into oligosaccharides and further into their monomeric sugars and sugar degradation products. Furthermore, other hemicellulosic hydrolysis products, such as acetic acid are also solubilyzed. The composition of the produced olive stones auto-hydrolysis liquors is presented in Table 1. Oligosaccharides are the main compounds present for every condition, followed by monomeric sugars and acetic acid. Furan derivatives, especially furfural arising from pentose degradation, are also present in relatively large concentrations.

The different composition of the liquors is a consequence of the different severities used for their production. Under the harsher conditions used to produce liquor 2, higher monosaccharide, aliphatic acids and furan derivatives concentrations are obtained, together with lower concentrations of soluble oligosaccharides, indicating a more extended hydrolysis.

Table 1
Composition (g/L) of olive stones auto-hydrolysis liquors

| | Compounds | Liquor 1 | Liquor 2 |
|---|---|---|---|
| Oligosaccharides | GOS | 0.62 | n.d. |
| | XOS | 28.72 | 27.99 |
| | AOS | 0.55 | n.d. |
| | AcOS | 4.84 | 4.88 |
| Monosaccharides | Glucose | n.d. | n.d. |
| | Xylose | 2.62 | 7.67 |
| | Arabinose | 1.06 | n.d. |
| Aliphatic acids | Formic acid | 0.36 | 1.03 |
| | Acetic acid | 1.96 | 5.64 |
| Furan derivatives | Hydroxymethyl furfural | 0.03 | 0.11 |
| | Furfural | 0.46 | 2.46 |

Liquor 1: *T* 200°C, LSR = 3.
Liquor 2: *T* 215°C, LSR = 4.
n.d. not detected.
AOS – arabino-oligosaccharides; GOS – gluco-oligosaccharides; XOS – xylo-oligosaccharides.

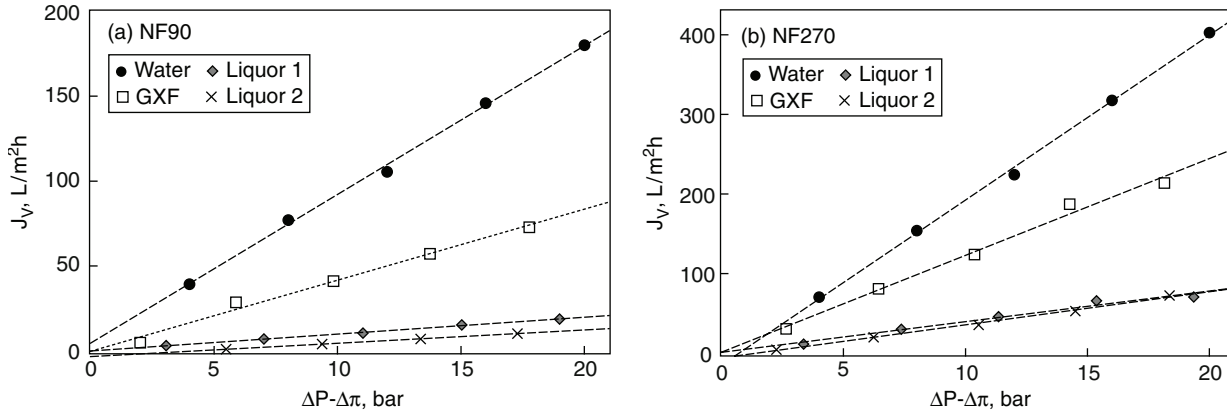


Fig. 2. Volumetric fluxes for water (circle), GXF solution (square) and liquor 1 (diamond) and 2 (cross) at different transmembrane pressures for the NF90 (a) and NF270 (b) membranes at 30°C.

### 3.2. Membrane permeability

Fig. 2 presents the experimental volumetric fluxes obtained for the NF90 and NF270 membranes at different transmembrane pressures, while in Table 2 the calculated permeability values are compiled.

The higher permeability values obtained for the NF270 membrane are a consequence of its higher MWCO. It may be observed that the processing of the GXF model solution lead to a significant decrease in permeability for both membranes, which may be attributed to a possible adsorption of furfural at the membrane surface, since glucose and xylose typically do not affect permeability. An even more significant decrease in permeability is observed during the processing of the auto-hydrolysis liquors. In this case, the complexity of the feed solution does not allow for a direct interpretation of the results as for the GXF model solution, but it may be tentatively assumed that the decrease in permeability is related to a larger variety of hydrophobic solutes present in the liquors that adsorb on the membrane surface. In all cases the membrane permeability was fully recovered by alkaline cleaning, which further indicates that the main fouling mechanism is a reversible adsorption of hydrophobic organic solutes on the membrane surface.

Table 2
Comparison between water, GXF solution and liquors 1 and 2 permeabilities for the NF90 and NF270 membranes at 30°C

| Membrane | $L_p$, l/m² hbar | | | |
|---|---|---|---|---|
| | Water | GXF | Liquor 1 | Liquor 2 |
| NF90 | 8.75 | 4.18 | 0.88 | 0.78 |
| NF270 | 20.69 | 12.16 | 3.85 | 4.17 |

### 3.3. Rejection of liquor components

The rejection to individual solutes present in the auto-hydrolysis liquors was determined in order to investigate the capability of the NF90 and NF270 membranes to be used in a diafiltration experiment. Fig. 3 shows the rejection profiles of both membranes for the GXF model solution. The results indicate that both membranes show a much higher rejection to glucose and xylose than to furfural. In the case of the NF90 membrane, even at low effective pressure differences ($\Delta P - \Delta\pi$) the rejection to glucose and xylose is higher than 95%. The NF270 membrane shows a lower rejection to these sugars, which can compromise its use in a diafiltration mode of operation.

Figs. 4 and 5 show the rejection results for liquor 1 and liquor 2, for the two NF membranes. In addition to glucose, xylose and furfural, in liquor 1 arabinose and acetic acid were detected in significant amounts, while in liquor 2 only acetic acid was additionally detected. Compounds detected only in small concentrations were not considered relevant to the objectives of this work, such as the case of small phenolics, acids (e.g., formic acid is detected in small quantities) or other sugars. Oligosaccharides of higher molecular weight (above 280 Da) were not considered in this study since they are highly rejected by both membranes. It should be additionally mentioned that, as the pH of both liquors were close to 3, acetic acid is expected to be in its neutral form ($pK_a = 4.76$) [17]. The results obtained for the liquors are similar to those obtained with the GXF model solution, where the NF90 membrane showed higher rejections to the target solutes, totally rejecting glucose, xylose and arabinose. It is interesting to note that the rejection profile of the NF270 membrane to acetic acid and furfural is uncommon, since the dependence of rejection with pressure is not monotonous. This could be a result of non-trivial interactions of the liquors solutes with the NF270

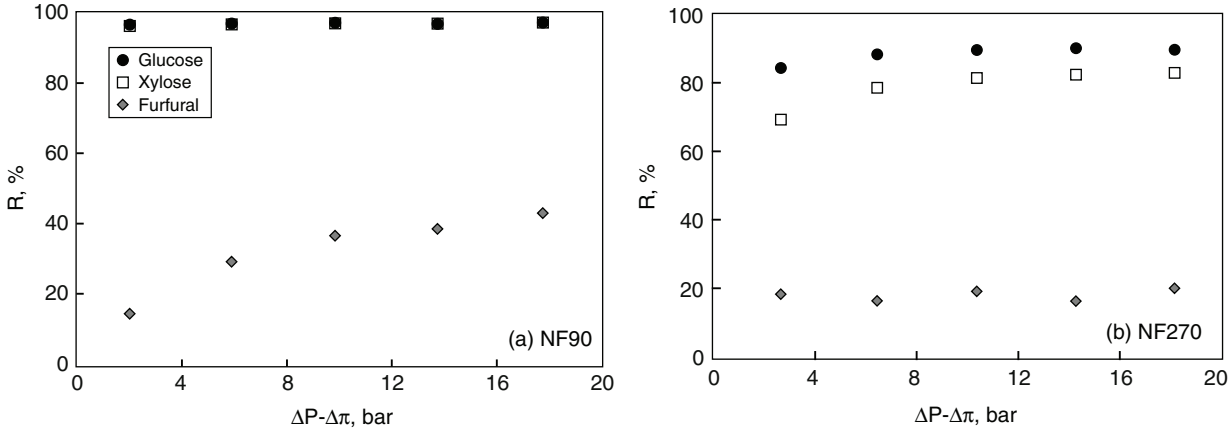


Fig. 3. Rejection profile for glucose (circle), xylose (square) and furfural (diamond) in the GXF model solution of (a) the NF90 membrane and (b) the NF270 membrane at 30°C.

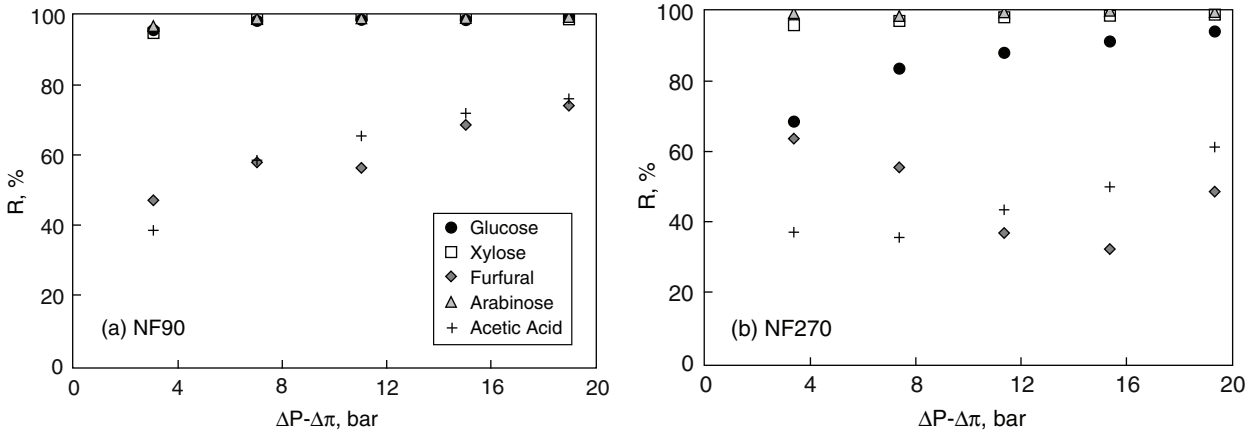


Fig. 4. Rejection profile for glucose (circle), xylose (square), furfural (diamond), arabinose (triangle) and acetic acid (cross) in liquor 1 of (a) the NF90 membrane and (b) the NF270 membrane at 30°C.

membrane material, but since its proprietary top-layer composition is not known, a further interpretation of the phenomena involved is behind the scope of this work. Therefore, the results suggest that the most adequate membrane for the aims of this work is the NF90, since it totally rejects both hexoses and pentoses, while allowing permeation of the potential inhibitory compounds, acetic acid and furfural. The NF270 membrane showed lower furfural and acetic acid rejection, but as the hexoses and pentoses rejection was not total, this behavior will eventually lead to a depletion of these sugars in a diafiltration mode of operation.

### 3.4. Diafiltration

The results obtained in the independent experiments using a total recycle mode of operation were used to design a diafiltration experiment to remove furfural and acetic acid from a liquor obtained from olive stone auto-hydrolysis at 215°C, liquor 2. The membrane selected for the diafiltration experiment was the NF90 membrane, since it showed a total rejection for hexoses and pentoses, and a moderate rejection for furfural and acetic acid. The applied pressure and temperature in the feed vessel were kept constant at 20 bar and 30°C, respectively. The total time of the experiment, considering the effective membrane area of the available module was chosen to be 5 d to allow furfural and acetic acid to be depleted to low values, based on a mathematical description of the diafiltration process. This involved the establishment of a mass balance over the feed recipient that may be given by:

$$\frac{\mathrm{d}}{\mathrm{d}t}\left(VC_{i,\mathrm{f}}\right) = -J_V A C_{i,\mathrm{p}} \tag{3}$$

By assuming that the volume in the feed recipient is constant, and that $C_{i,\mathrm{p}} = C_{i,\mathrm{f}}(1 - R_i)$, manipulation of the previous equation gives:

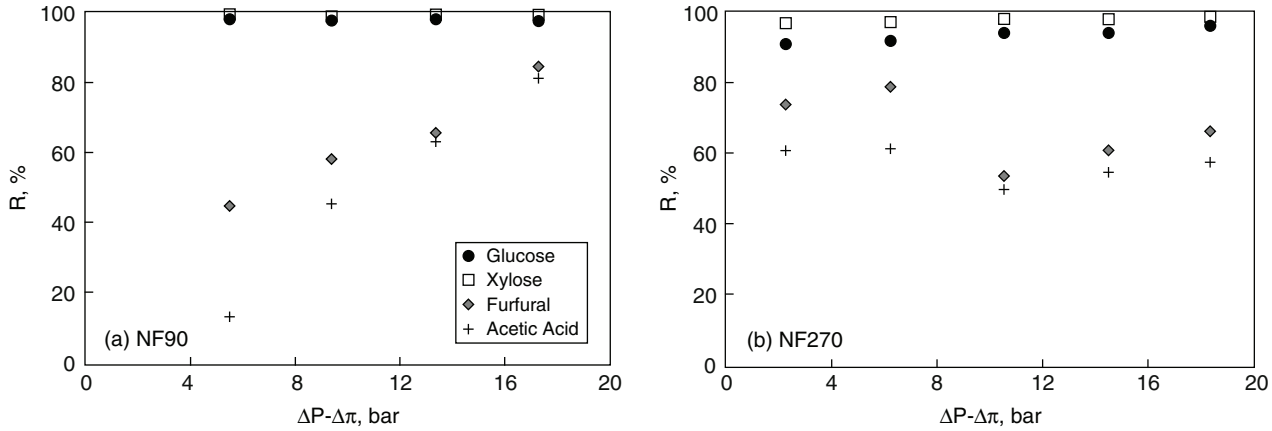


Fig. 5. Rejection profile for glucose (circle), xylose (square), furfural (diamond) and acetic acid (cross) in liquor 2 of (a) the NF90 membrane and (b) the NF270 membrane at 30°C.

$$\frac{dC_{i,f}}{dt} = \frac{-J_V A C_{i,f}}{V}(1-R) \quad (4)$$

The rejection is typically assumed to be constant for uncharged solutes, therefore the previous equation may be solved algebraically to give:

$$C_{i,f} = C^0_{i,f} \exp\left(\frac{-J_V A C_{i,f}(1-R)}{V} t\right) \quad (5)$$

The rejection of xylose, glucose, furfural and acetic acid in the model equation was fixed at the values obtained in the previous experiments (please see Fig. 5), and the NF90 membrane permeability was assumed to be constant and equal to 0.78 l/m² hbar (please see Table 1). Fig. 6 shows the evolvement over time of the concentration of the target solutes in the diafiltration feed recipient. The agreement between predicted and experimental values can be considered satisfactory taking into account the complexity of the liquor composition. In this experiment, the xylose and glucose content was kept constant as expected, while furfural and acetic acid were significantly depleted. It is important to note that, in the case of furfural, the concentration of the last experimental point was below the limit of detection of the analytical method used. Fig. 6 includes the number of diafiltration volumes (ratio of washing solution volume to feed volume) as well, needed for a given depletion. It may be observed that over seven diafiltration volumes were processed during the 5 d diafiltration experiment, which enabled a 50% decrease in the acetic acid concentration, and an almost total removal of furfural.

As a result of its validation with experimental data, the predictive model can be applied for the simulation of different operating conditions than those used in the diafiltration experiment. Fig. 7 shows the depletion of furfural and acetic acid as a function of the number of

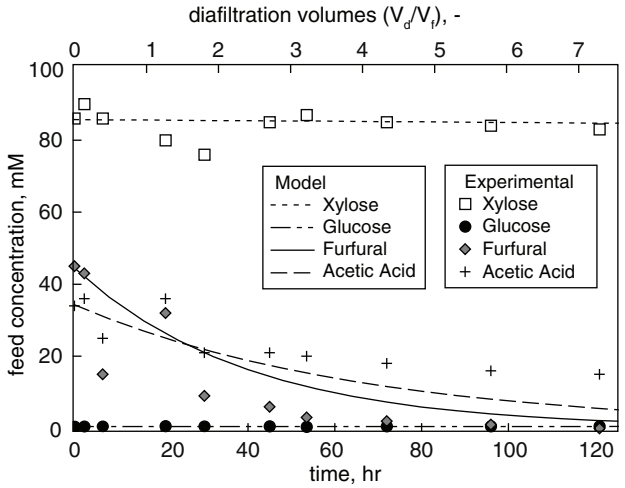


Fig. 6. Diafiltration feed recipient content as a function of time.

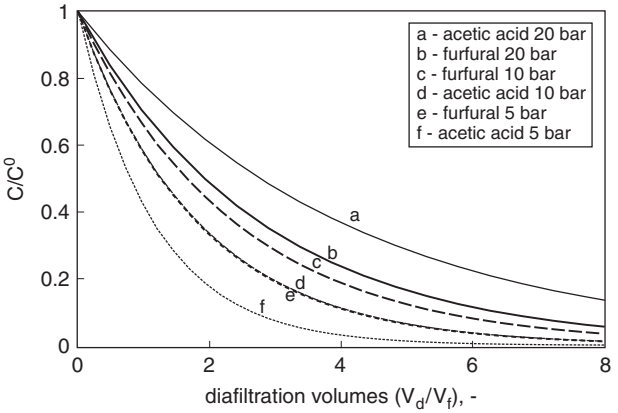


Fig. 7. Sensitivity analysis of furfural and acetic acid depletion as a function of the number of diafiltration volumes for different transmembrane pressures.

diafiltration volumes, for varying transmembrane pressures. A change in transmembrane pressure will affect not only the permeate flux, but also the solute rejection, as shown in Figs. 4 and 5, where the rejection is typically lower at lower pressures. The simulation results indicate that working at a lower transmembrane pressure leads generally to a more efficient washing of furfural and acetic acid from the feed recipient in terms of diafiltration volumes.

## 4. Conclusions

The primary objective of this work was the development of a nanofiltration process in diafiltration mode for the separation of sugars from metabolic inhibitors produced during auto-hydrolysis of olive stones. Both membranes tested, NF90 and NF270, enabled a partial separation of sugars from the metabolic inhibitors, but the NF90 membrane was shown to be the most efficient by presenting a higher sugar rejection.

A diafiltration experiment was carried out for several days using real hydrolysate liquor in order to test the assumptions made. The results showed that the mathematical model developed in this work agreed very well with the experimental diafiltration data, thus it can be applied as a cost-effective solution for the evaluation of optimized operating conditions and subsequent scale-up.

The application of this process to the purification/detoxification of auto-hydrolysis liquors seems promising since a complete separation of furan derivatives/furfural and acetic acid may be achieved provided the number of required diafiltration volumes is met.

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## Symbols

$J_V$ — Solvent volumetric flux
$L_P$ — Membrane permeability
$P$ — Pressure
$\Pi$ — Osmotic pressure, $\pi = RTC$
$R_i$ — Rejection of solute $i$
$C_{i,P}$ — Concentration in the permeate of solute $i$
$C_{i,f}$ — Concentration in the feed of solute $i$
$V$ — Volume in the feed recipient
$A$ — Membrane area

## References

[1] G. Rodríguez, A. Lama, R. Rodríguez, A. Jiménez, R. Guillén and J. Fernández-Bolaños, Bioresour. Technol., 99 (2008) 5261–5269.
[2] F. Carvalheiro, T. Silva-Fernandes, L.C. Duarte and F.M. Gírio, Appl. Biochem. Biotech., 153 (2009) 84–93.
[3] C. Cara, M. Moya, I. Ballesteros, M.J. Negro, A. González and E. Ruiz, Process Biochem., 42 (2007) 1003–1009.
[4] I. Ballesteros, J.M. Oliva, M.J. Negro, P. Manzanares and M. Ballesteros, Appl. Biochem. Biotech., 98–100 (2002) 717–732.
[5] I. Ballesteros, J.M. Oliva, M.J. Negro, P. Manzanares and M. Ballesteros, Grasas Aceites, 53 (2002) 282–288.
[6] J. Fernández-Bolaños, G. Rodríguez, E. Gómez, R. Guillén, A. Jimenez, A. Heredia and R. Rodríguez, J. Agri. Food Chem., 52 (2004) 5849–5855.
[7] A. Moure, P. Gullón, H. Domínguez and J.C. Parajó, Process Biochem., 41 (2006) 1913–1923.
[8] P. Christakopoulos, P. Katapodis, E. Kalogeris, D. Kekos, B.J. Macris, H. Stamatis and H. Skaltsa, Int. J. Biol. Macromol., 31 (2003) 171–175.
[9] M.P. Esteves, F. Carvalheiro, P. Moura, M.T. Amaral-Collaço and F.M. Gírio, in: Use of arabino-xylo-oligosaccharides from brewery's spent grains as growth substrate for probiotic strains, R. Lásztity, W. Pfannhauser, L. Simon-Sarkadi and S. Tömösközi, eds. European Conference on Functional Foods – a New Challenge for the Food Chemist, Euro Food Chem X Budapeste, 1999, 96–100.
[10] P. Gullón, P. Moura, M.P. Esteves, F.M. Gírio, H. Domínguez and J.C. Parajó, J. Agr. Food Chem., 56 (2008) 7482–7487.
[11] P. Moura, R. Barata, F. Carvalheiro, F.M. Gírio, M.C. Loureiro-Dias and M.P. Esteves, LWT 40 (2007) 963–972.
[12] F. Carvalheiro, L.C. Duarte, S. Lopes, J.C. Parajó, H. Pereira and F.M. Gírio, Process Biochem., 40 (2005) 1215–1223.
[13] R. Vegas, J.L. Alonso, H. Domínguez and J.C. Parajó, J. Agr. Food Chem., 52 (2004) 7311–7317.
[14] B. Han, W. Carvalho, L. Canilha, S.S. da Silva, J.B.A. E. Silva, J.D. McMillan and S.R. Wickramasinghe, Desalination, 193 (2006) 361–366.
[15] P. Gullón, M.J. González-Muñoz, H. Domínguez and J.C. Parajó, J. Food Eng., 87 (2008) 257–265.
[16] R. Vegas, A. Moure, H. Domínguez, J.C. Parajó, J.R. Alvarez and S. Luque, Bioresour. Technol., 99 (2008) 5341–5351.
[17] Y.H. Weng, H.J. Wei, T.Y. Tsai, W.H. Chen, T.Y. Wei, W.S. Hwang, C.P. Wang and C.P. Huang, Sep. Purif. Technol., 67 (2009) 95–102.
[18] Y.H. Weng, H.J. Wei, T.Y. Tsai, T.H. Lin, T.Y. Wei, G.L. Guo and C.P. Huang, Bioresour. Technol., 101 (2010) 4889–4894.
[19] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton and D. Crocker, Determination of Structural Carbohydrates and Lignin in Biomass, NREL, 2005.