Process Biochemistry 49 (2014) 173–180

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Detoxification of hemicellulosic hydrolysates from extracted olive pomace by diananofiltration

Teresa Brás[a,b], Vera Guerra[c], Ivone Torrado[c], Pedro Lourenço[d], Florbela Carvalheiro[c], Luís C. Duarte[c], Luísa A. Neves[a,e,*]

[a] Centro de Biotecnologia Agrícola e Agro-Alimentar do Baixo Alentejo e Litoral (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal
[b] CICECO, University of Aveiro, 3810-193 Aveiro, Portugal
[c] Unidade de Bioenergia-LNEG, Ed. K2, Estrada do Paço do Lumiar, 22, 1649-038 Lisboa, Portugal
[d] UCASUL, Rua Mira Fernandes, 2, 7801-901 Beja, Portugal
[e] REQUIMTE/CQFB, FCT, Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

ARTICLE INFO

Article history:
Received 6 June 2013
Received in revised form
10 September 2013
Accepted 11 September 2013
Available online 25 September 2013

Keywords:
Nanofiltration
Diafiltration
Hydrolysate detoxification
Fermentation inhibitors
Sustainable membrane processing

ABSTRACT

Xylitol can be obtained from the pentose-rich hemicellulosic fraction of agricultural residues, such as extracted olive pomace, by fermentation. Dilute acid hydrolysis of lignocellulosic materials, produces the release of potential inhibitory compounds mainly furan derivatives, aliphatic acids, and phenolic compounds. In order to study the potential on the increase of the hydrolysate fermentability, detoxification experiments based on diananofiltration membrane separation processes were made. Two membranes, NF270 and NF90, were firstly evaluated using hydrolysate model solutions under total recirculation mode, to identify the best membrane for the detoxification. NF270 was chosen to be used in the diananofiltration experiment as it showed the lowest rejection for toxic compounds and highest permeate flux. Diananofiltration experiments, for hydrolysate model solutions and hydrolysate liquor, showed that nanofiltration is able to deplete inhibitory compounds and to obtain solutions with higher xylose content. Conversely to non-detoxified hydrolysates, nanofiltration detoxified hydrolysates enabled yeast growth and xylitol production by the yeast *Debaryomyces hansenii*, clearly pointing out that detoxification is an absolute requirement for extracted olive pomace dilute acid hydrolysate bioconversion.

1. Introduction

In the Mediterranean area, olive tree is a very widespread cultivar, and olives represent one of the most important agriculture products [1–3]. In the Mediterranean area, the production of olives has been estimated at 7.8×10^6 ton [4] where olive pomace represents approximately 35% (w/w) of the processed olives [1]. This material can undergo solvent extraction to yield olive pomace oil and extracted olive pomace. The later, has no significant industrial use and must be upgrade. Likewise, to other agro-food residues, it is a lignocellulosic residue, whose hemicellulose fraction yields significant amounts of xylose, under acidic conditions.

The resulting liquid phase from acid hydrolysis, the hemicellulosic hydrolysate, can be used in fermentation media suitable for xylitol production [5], a commercial polyol with many applications in food, cosmetics and pharma industries [6].

During acid hydrolysis, several fermentation inhibiting and toxic compounds are also released and produced. These compounds may be divided in three groups, namely, furan derivatives, aliphatic acids and phenolic compounds [7–10]. Furans, furfural and hydroxymethylfurfural (HMF), are the result of pentose and hexose degradation during hydrolysis. Both can inhibit cells affecting mainly the duration of the lag phase [11,12]. Formic and levulinic acids are formed from furans degradation [10] and together with the quantitatively more relevant acetic acid, derived from the release of acetyl groups present in the hemicelluloses, are the main aliphatic acids present. Their inhibitory potential varies depending on the concentration of the undissociated form and cultivation conditions employed in the fermentative process [5,9–11]. Phenolic compounds, derived both from extractives or from lignin degradation, have also a potential inhibitory effect on the fermentation of lignocellulosic hydrolysates, causing a partition and loss of integrity of biological membranes [8]. All these compounds can also interact synergistically with each other, further stressing the fermentation [13–15].

In order to remove the inhibitors and increase the hydrolysate fermentability, several detoxification treatments have already been studied [7,10]. The main option is typically the use of over-liming

* Corresponding author at: REQUIMTE/CQFB, FCT, Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal. Tel.: +351 212948385; fax: +351 212948550.
E-mail address: lan11892@fct.unl.pt (L.A. Neves).

1359-5113/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.procbio.2013.09.017

and/or active charcoal treatments, together with evaporation [16], that enables to remove the most volatile compounds such as furfural. Nevertheless, more recently, alternative processes such as the use of ion-exchange resins [17,18] and reactive membrane extraction [19], have been shown to present some advantages, although they are still somewhat expensive.

An alternative to those detoxification treatments may be the use of the pressure driven membrane process such as nanofiltration. Han et al. [20,21] were the first to study the separation of acetic acid from sugars and more recently some works have already studied the separation of carboxylic acids and furans from hydrolysates of rice straw, bio-oils or lignocellulosic hydrolysate model solutions [22–27].

Although there are some studies about lignocellulosic acid dilute hydrolysates, this work presents a new approach. Conversely to the literature, that is focused on the removal of one inhibitor or on the modeling, we are interest on the total removal of inhibitors prior to the fermentation. Therefore, it is important to study the detoxification of extracted olive pomace hydrolysates, using diananofiltration with real hydrolysates.

Santos et al. [28], studied the use of nanofiltration to remove inhibitory compounds from olive stones autohydrolysis liquors and showed that nanofiltration has a great potential to reduce the inhibitory compounds concentration, retaining the xylo-oligosaccharides.

In this work, the use of nanofiltration under diafiltration mode was studied as a detoxification method to remove inhibitory compounds from extracted olive pomace diluted acid hydrolysate and improve its xylose fermentability by *Debaryomices hansenii*.

2. Materials and methods

2.1. Raw material and hydrolysate preparation

Sulfuric acid (95–97%), Glucose (≥99.5%), xylose (≥99%), arabinose (≥99%) and hydroximethylfurfural (HMF) (≥99%) were obtained from SIGMA (Germany). Furfural (≥99%) was obtained from FLUKA (Switzerland). Formic (98–100%) and Levulinic acids (for synthesis) were obtained from Merck (Germany). Acetic acid (99.7%) was obtained from BDH Prolabo (France).

The extracted olive pomace used in this work was kindly provided by UCASUL (Alvito, Beja, Portugal) and was collected in the 2010/2011 campaign. The extracted olive pomace was sieved to retain particles between 1.00 and 3.55 mm, homogenized into a defined lot and stored at room temperature in plastic containers. Dilute acid hydrolysis was carried out as optimized before [29] using 3.5% Sulfuric acid, at a liquid to solid ratio of 3 and 130 min isothermal period. After hydrolysis, the hydrolysate is kept frozen until use. Before nanofiltration, hydrolysate was pre-filtered with a filter of 0.45 μm from Pall (U.S.A.) and pH was corrected to 3 with NaOH pellets from Merck (Germany), according to experiments made with model solutions.

2.2. Nanofiltration experiments

Taking into account the satisfactory results obtained by Santos et al. [28] on the removal of inhibitory compounds from olive stones autohydrolysis liquors, using NF270 and NF90 membranes and due to their main characteristics shown in Table 1, these two commercial membranes, NF270 and NF90 were used.

2.2.1. Experimental setup

The nanofiltration experimental setup used in this work is shown in Fig. 1. It is comprised by a GE-Sepa CF cross-flow module (GE Osmonics, USA) and a high pressure feed pump (Hydra-cell model G13, Wanner Engineering Inc., USA). The effective membrane area used was 140 cm^2.

Table 1
Characteristics of NF90 and NF270 membranes.

	NF90	NF270
Manufacturer	Dow/Filmtec	Dow/Filmtec
Surface material	Polyamide	Polyamide
Maximum temperature (°C)	45	45
Maximum pressure (bar)	41	41
Molecular Weight cut-off (Da)	100 [28]	400 [28]
Average pore diameter	0.68 [48]	0.84 [48]
Isoelectric point	4 [34]	4-5[39]

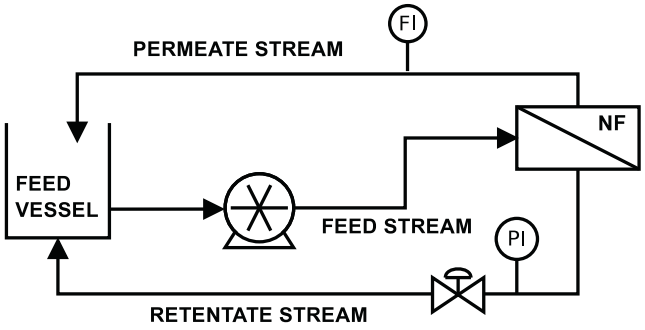

Fig. 1. Experimental nanofiltration setup (NF). PI and FI are pressure and flow-rate indicators, respectively.

Table 2
Composition of the model solution used for diafiltration optimization tests.

Compounds	C_s (g/L)
Glucose	2.3
Xylose	37.3
Arabinose	3.4
Formic acid	2.1
Acetic acid	13.7
Levulinic acid	0.4
HMF	0.5
Furfural	3.6

In order to get a homogenized membrane, before starting the experiments, each new membrane was compacted with deionized water at 20 bar until a constant value of flux was obtained. During membrane compactation the system was operated under a total recycle mode of operation, in which both the retentate and the permeate streams were recirculated to the feed recipient. The hydraulic permeability value for all the membranes tested was measured, after compaction, at a pressure range between 4 and 20 bar, at 34 °C (average temperature) using deionized water.

2.2.2. Operational modes

Due to the complexity of hemicellulosic hydrolysate, nanofiltration experiments were firstly made using a model solution similar to hydrolysate composition itself for sugars, aliphatic acids and furans (described in Table 2).

The nanofiltration tests were divided in two phases: a total recirculation mode and a diafiltration mode. In order to obtain the optimal operation conditions (transmembrane pressure, pH and membrane) and to get the data necessary to the theoretical prediction of diafiltration experiment, total recirculation mode was prior made to diafiltration.

(a) Total recirculation mode (TRM): A volume of 2 L of model solution was prepared. In this configuration, both the retentate and permeate streams were recirculated to the feed recipient. A transmembrane pressure between 4 and 20 bar was applied, and a recirculation flow rate of 300 L/h was used. Two pH values of the feed solution were tested, 5.5 and 3. Permeate, retentate and feed samples were collected for each experimental condition.
(b) Diafiltration mode: For the optimal conditions obtained on TRM mode, a diafiltration experiment was also carried out in order to optimize the rejection of sugars. Since the detoxified hydrolysate is going to be used for the production of xylitol and although xylose presents a high rejection for the studied membranes, on a diafiltration step there will be some losses. In order to minimize this effect, a pre and post concentration was considered and the total diafiltration experiment comprised three different phases, as explained below.
   a. Pre-concentration mode (PCM): A volume of 5 L of model solution was prepared. The permeate was continuously removed at a constant pressure of 20 bar and a recirculation flow of 300 L/h was used. Samples of permeate, retentate and feed were collected when 500, 1000, 1500, 2000 and 2500 mL of the collected permeate was reached.
   b. Diafiltration (D): Permeate was continuously removed and feed volume was hold with the addition of deionized water. Samples of feed and permeate were collected periodically during the experiment. For the model solution a volume of 9 L of deionized water was added.
   c. Post-concentration mode (PDM): The permeate was continuously removed at a constant pressure of 20 bar and a recirculation flow of 300 L/h was used. Samples of permeate and feed were collected when 250, 500, 750, 1000, 1250 and 1500 mL of the collected permeate was reached.