

3.3 Hemp hurds as sugar source for ABE fermentation with *in-situ n*-butanol recovery

Unpublished results

Introduction

The use of inexpensive lignocellulosic residues as a carbon source for fermentative production *n*-butanol is seen as one of the most promising driver to increase the overall economy of the process. An interesting feature of the ABE fermentation is that most Clostridia are able to utilize different carbohydrates (including pentoses such as xylose) [1]. Currently, *n*-butanol is an important platform chemical used as a solvent or converted to acrylates, ethers and butyl acetate. In addition, it is promise as a second-generation biofuel, thanks to its higher combustion value than ethanol and its favorable blending properties. *N*-butanol is considered a genuine "drop-in" biofuel. It provides a viable commercial market outlet that allows for bio-butanol production to gain a foothold [2]. Despite that, fermentative production of *n*-butanol suffers of low product accumulation and low space–time-yields due to its toxicity to cells.

Membrane-based methods, such as pervaporation are promising product recovery and removal techniques. In a pervaporation process a liquid feed stream, such as a fermentation broth, is brought into contact with one side of a non-porous membrane while a vacuum or gas purge is applied to the other side. Certain compounds of the feed liquid, according to their sorption and diffusion behaviors with the membrane, can be enriched into the permeate [3]. The effectiveness of pervaporation is measured by two parameters: the selectivity (α) and flux (J), which in turn are dependent to the membrane

material and process conditions (e.g. feed composition and concentrations, temperature and membrane surface area and thickness). All these features dictate the separation and productivity achieved by the process. [4].

In this study the fermentability of hemp hurds derived C6 and C5 sugars streams, was investigated for the production of *n*-butanol using *C. acetobutylicum*. The pervaporation process, by using organophilic membranes, was considered for the *in-situ* solvents recovery. In this regard, the effect of temperatures and membrane surface area on the flux and selectivity was evaluated using model butanol/acetone/ethanol water mixtures.

Material and methods

Culture and inoculum preparation

C. acetobutylicum strain ATCC 824 (Belgian co-ordinated collections of micro-organisms, BCCM) was used. Freeze-dried cultures supplied in vacuum-sealed ampoules were used to prepare thioglycolate culture media. After inoculation and incubation at 37 °C for 72 h, 1.67 mL 80% glycerol solution was added per mL of culture. The culture was divided in 1.5 mL vials and subsequently stored as stock culture at -20 °C. Anaerobic shake flasks containing 100 mL of a defined medium solution were inoculated with 0.1 mL of the glycerol stock culture and incubated at 35 °C for 28 h to obtain cultures in the mid-exponential phase (pH 4.5). The medium used for the seed culture and the fermentations were identical and contained (for 1 L) 0.01 g NaCl, 2.2 g ammonium acetate, 0.5 g K₂HPO₄, 0.5 g KH₂PO₄, 0.01 g biotin, 1 mg *p*-aminobenzoic acid (PABA), 0.2 g MgSO₄ 7H₂O, 0.01 g MnSO₄ H₂O, 11.1 mg NH₄Fe citrate and 60 g glucose or xylose. The medium containing all components except glucose, biotin and PABA was autoclaved at 121 °C for 15 min. A concentrated solution of glucose was separately autoclaved, biotin and PABA were sterile filtered and added to the rest of the medium before inoculation.

Hemp hurds C6 and C5 sugars streams and fermentation.

The procedure used to produce the C6 and C5 hemp hurds derived sugars streams (C6-HH and C5-HH, respectively) and its characterizations are described in Gandolfi et al. [5]. Batch fermentation of C6-HH and C5-HH and controls were carried out in 1.5 L, using a 10% v/v preculture inoculum. Fermentors, equipped with oxygen, OD and pH sensors, were sparged with N₂ prior to inoculation and fermentations were allowed to proceed anaerobically for 48 h at 37 °C, without pH control. The cultures were periodically sampled for cell quantification and metabolite characterization.

Pervaporation

The composition of the ternary ABE model solution was: butanol 12 g L⁻¹, acetone 6 g L⁻¹ and ethanol 2 g L⁻¹. The in-house developed pervaporation unit composed by three identical polydimethylsiloxane (PDMS) membrane, consisting of a separating layer of approximately 1 μm thickness on top of a porous polyimide support (approximately 200 μm) were connected in series with an effective surface area of 0.009 m² each (A). The temperature of the heating bath in the recirculation coil was set at 37 or 50 or 60 °C. Peristaltic pumps were used for the feed (1.4 L h⁻¹) and for recirculation (30.6 L h⁻¹). After reaching the desired temperature a permeate pressure of 15-20 mbar was established using a membrane vacuum pump. Once the steady state was obtained, samples of both retentate and permeate were collected for analysis and total solvent recovered in the permeate were weighed (W). The permeate flux (J) was obtained by Eq. 1, while the selectivity factor (α_{i,w}) was calculated according to Eq. 2.

$$J = \frac{W}{A \cdot t} \quad (1)$$

$$\alpha_{i,w} = \frac{\omega_i^p / \omega_w^p}{\omega_i^f / \omega_w^f} \quad (2)$$

ω^p and ω^f are the weight fractions of solutes in permeate and feed, respectively, while the sub-index *i* refer to organic and *w* refers to water.

Analysis

After filtration, 0.2 mL of cultures sample was added to 1.8 mL water and 500 μL of 72% w/v H_2SO_4 . Subsequently, 80 μL of an aqueous solution containing 6 mg L^{-1} of 2-methylhexanoic acid was added as internal standard. A small amount of NaCl was added together with 2 mL diethylether for extraction of the Volatile Fatty Acids (VFAs). The sample was vortexed and subsequently centrifuged. The supernatant was transferred to a vial for analysis by gas chromatography with flame ionization detection. The determination of acetone, butanol ethanol, acetic acid and butyric acid was carried out by gas chromatography with flame ionization detection. D6-ethanol was used as an internal standard. The concentrations of glucose and xylose were determined by high performance liquid chromatography with evaporative light scattering detector.

Results and discussion

Operating the bioconversion continuously has several advantages over batch and fed-batch operations. Among them shortening the equipment downtime and avoiding the time loss due to the lag phase of the microbial culture are the most important issues [1]. The choice for a two-stage operation results from the observation that *C. acetobutylicum* and many other Clostridia strains are present in two distinct metabolic states, i.e. the acidogenic and the solventogenic one [6]. Moreover, the two-stage continuous operation coupled with *in-situ* product removal, increases the production space-time, solvent productivities and therefore allows obtaining high solvent yields.

Fermentation of lignocellulosic sugars

Prior to carrying out the continuous two-stage operation, investigations were made to ascertain the ability of *C. acetobutylicum* ATCC 824 to ferment the sugars streams obtained from hemp hurds. To this aim, parallel batch fermentation of commercial glucose or xylose (control) and C6-HH or C5-HH, were carried out at initial sugar level of 60 g L^{-1} . In the control

experiment using glucose the best ABE titer achieved was 3.83 g L⁻¹. This value is far from the solvent concentration of about 20 g L⁻¹, usually observed fermenting this strain [1]. Similar unsuccessful results (4.29 g L⁻¹ of total ABE) were obtained for using C6-HH sample. Fermentation results for control glucose and C6-HH as carbon source are given in Tab. 1.

	Control #1	Control #2	C6-HH #1	C6-HH #2
Acetic acid (g L ⁻¹)	3.04	3.00	3.82	3.40
Lactic acid (g L ⁻¹)	3.09	1.95	3.01	1.84
Butyric acid (g L ⁻¹)	1.06	3.04	1.69	2.97
Acetone (g L ⁻¹)	0.66	1.65	0.62	1.65
Butanol (g L ⁻¹)	1.19	2.06	1.28	2.51
Ethanol (g L ⁻¹)	0.11	0.12	0.09	0.13
Glucose uptake (%)	23.8	43.8	29.2	37.4
ABE yield (%)	12.6	16.0	8.3	17.0

Table 1. *C.acetobutylicum* ATCC 824 fermentation results of two independent experiments (#1 and #2).

Suboptimal glucose uptake, acid generation and solvent production have been obtained, thus indicating possible strain instability or media issue. Moreover the possibility of strain solvent degeneration, described by different authors, was not considered, as the acids accumulation in the media was low (Tab. 1) [7]. Despite that, control and C6-HH fermentations showed similar behaviors in terms of biomass grow and sugar consumption, suggesting the fermentability of the biomass-derived sugar sample C6-HH. Fermentations profiles are shown in Fig 1. Additionally, no cell growth was observed when xylose (control) and C5-HH were used as carbon source for

fermentation. Again this result indicated some problems related to the strain or to the media.

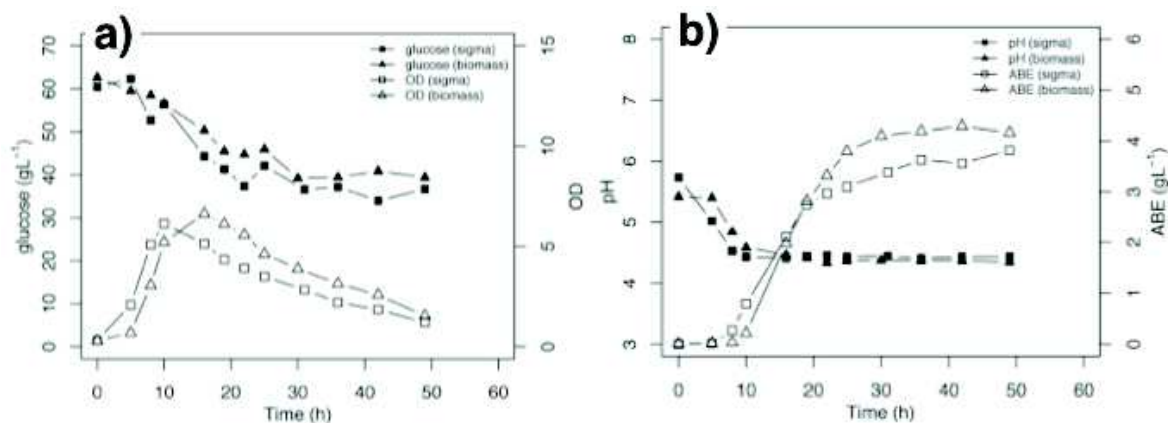


Figure 2. Fermentation profiles of Glucose (control) squares and C6-HH (sample) triangles. Time course of glucose uptake and biomass grow (a). Time course of pH and total solvent production (b).

Pervaporation of model ABE solution

The effect of operating temperature on pervaporation performance of the PDMS membrane is illustrated in Fig. 2. The total flux increases from 694 to 2150 g m⁻²h⁻¹ as the temperature increases from 30 to 60 °C (Tab. 2). When the temperature increases, the PDMS swells and the polymer segments have more free volume and chain mobility. Moreover, the vapor pressure difference is higher, enhancing the transport driving force. Both factors favor the diffusion of molecules through the PDMS membrane, leading to higher permeate flux J . Increasing membrane surface area, from 0.018 to 0.027 m² seems to not strongly affect the fluxes as temperature do. Conversely to the flux, the butanol selectivity factor increase as function of both temperature and surface area (Tab. 2).

Temperature (°C)	Membrane area (m ²)	Flux (g m ⁻² h ⁻¹)	α Acetone/H ₂ O	α BuOH/H ₂ O	α EtOH/H ₂ O
37	0.018	655	15.18	12.11	4.90
37	0.027	694	16.33	11.49	5.28
50	0.018	1410	14.22	12.98	5.50
50	0.027	1320	16.80	15.58	5.56
60	0.018	2320	15.04	15.75	5.80
60	0.027	2150	15.18	15.88	6.15

Table 2. Pervaporation results using the ABE model ternary solution.

Conclusion

The results obtained showed that *C. acetobutylicum* potentially is able to ferment lignocellulosic-derived sugars for the production of *n*-butanol. This is of importance as inexpensive and renewable carbon source could be used coupled with advanced fermentation scheme and *in-situ* product removal, allowing obtaining a cost-effective production process for this useful platform chemical.

This work has been carried out at the Flemish Institute for Technological Research under the framework project COST CM 0903 (Utilisation of Biomass for Sustainable Fuels & Chemicals - UBIOCHEM). Due to the time constrain and substrate shortage, has not been possible to repeat the ABE fermentation tests after the problem related to poor microorganism growth has been fixed by the host laboratory.

