Biobutanol production from apple pomace: The importance of pretreatment methods on the hydrolysis of lignocellulosic agro-food wastes

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Abstract

- Apple pomace was used as a feedstock for biobutanol production (ABE fermentation) with *Clostridium beijerinckii* CECT 508.
- The biomass was pretreated with five different soft physicochemical methods (autohydrolysis, acids, alkalis, organic solvents and surfactants) and was subsequently subjected to an enzymatic treatment to complete the hydrolysis.
- The pretreatment was optimized to maximize the amount of simple sugars released and to minimize the generation of fermentation inhibitors.
- The best pretreatment (PEG 6000 1.96 % w/w, 100 ºC, 5 min) produced a hydrolysate with 42 g/L sugars and low concentrations of inhibitors.
- The hydrolysate was fermented by *Clostridium beijerinckii* CECT 508 in 96 h (9.11 g/L butanol, 0.276 g_B/g_S) without the need of a detoxification stage.
- Therefore, apple pomace could be a suitable feedstock for biobutanol production via ABE fermentation.

Introduction and aims of the study

Apple pomace is a solid waste generated after milling and processing apples in juice industries. Due to its high proportions of cellulose and hemicellulose, apple pomace could be a suitable feedstock for butanol biorefineries. Solventogenic bacteria used for ABE fermentation are not able to directly ferment polysaccharides. For this reason, a cost-effective pretreatment of the biomass followed by an enzymatic hydrolysis is needed to release simple sugars.

The objective of this research is to find an adequate pretreatment method for apple pomace in order to perform an ABE fermentation of its hydrolysates with *C. beijerinckii* CECT 508. Five different soft physico-chemical pretreatments (autohydrolysis, acids, alkalis, organic solvents and surfactants) were compared; focusing on the production of high sugar concentrations and low inhibitor concentrations.

Material and methods

Biomass description

Dry apple pomace was ground and sieved to a size of 0.5-1.0 mm (Fig. 1).

Pretreatment

Preliminary tests were performed with various reagents of the following groups: acids, alkalis, organic solvents and surfactants. Finally, HNO_3 (acid), acetone (organic solvent) and PEG 6000 (surfactant) were chosen together with pure water (autohydrolysis) to perform optimisation tests. Pretreatments were carried out in a high-presure 2-L reactor made of alloy Carpenter-20 (Fig. 2), whose working parameters (temperature, time and reagent concentration) were optimised via RSM experimental design. The biomass-to-solvent ratio was 10% w/w.

Enzymatic hydrolysis

The whole slurry obtained in the pretreatment was subjected to hydrolysis with the enzymatic cocktail Cellic CTec2 (pH 5.0, 50 ºC, 180 rpm, 72 h).

Fermentation

The hydrolysates were filtered and supplemented with nutrients (5 g/L yeast extract, 2.1 g/L NH_4Cl, 0.5 g/L K_2HPO_4, 0.5 g/L KH_2PO_4, 0.01 g/L $FeSO_4 \cdot 7H_2O$, 0.2 g/L $MgSO_4 \cdot 7H_2O$, and 0.5 g/L cysteine) to perform ABE fermentation with *C. beijerinckii* CECT 508, at 35 ºC, 100 rpm, initial pH of 6.0 (contolled by $CaCO_3$) and anaerobic conditions during 96 h (Fig. 3).

Fig. 1. Dry apple pomace.

Fig. 2. High-pressure reactor.

Fig. 3. ABE fermentation.

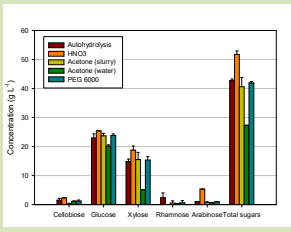
Results

Pretreatment optimization

Optimal working parameters for each pretreatment are shown in Table 1. Sugar and inhibitor concentrations depended on the physicochemical pretreatment applied (Fig. 4).

	Pretreatment optimal conditions (RSM)		
	T (°C)	t (min)	Reagent (%, w/w)
Autohydrolysis	142.4	12.0	-
HNO_3	124.2	7.3	1.83
Acetone	112.1	5.0	10
PEG 6000	100.2	5.0	1.96

Table 1. Optimal working conditions for each pretreatment.



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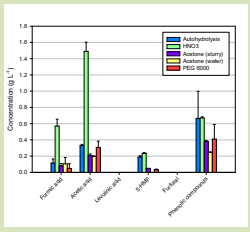

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Fig. 4. Composition of apple pomace hydrolysates for each pretreatment under optimal conditions. a) Released sugars. b) Inhibitors generated. (*) Concentrations measured after pretreatment + enzymatic hydrolysis.

Fermentability

All the hydrolysates tested were fermentable, except that of nitric acid. The hydrolysates from autohydrolysis and PEG 6000 pretreatment produced the highest butanol concentrations (Table 2).

Table 2. Sugar consumption and ABE parameters for a 96-h fermentation of apple pomace hydrolysates by *C. beijerinckii* CECT 508.

	Sugar consump. (%)	ABE metabolites (g/L)					Yield	Productivity
	Total	Acetone	Butanol	Ethanol	Acetate	Butyrate	Y_B (g/g)	W_B (g/L·h)
Autohyd.	91±3	1.9±0.4	6.3±1.0	0.2±0.0	3.9±0.4	4.0±0.4	0.17±0.02	0.07±0.01
HNO_3	6±1	0 .0±0.0	0.1±0.0	0 .0±0.0	1.6±0.1	1.3±1.5	0.03±0.00	0.00±0.00
Acetone	96±1	8.6±0.2	5.1±0.2	0.2±0.0	2.4±0.0	0.9±0.1	0.19±0.01	0.05±0.00
PEG 6000	91±0	3.6±0.0	9.1±0.2	0.3±0.0	1.8±0.1	0.8±0.0	0.28±0.01	0.09±0.00

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