Industrial potato peel as a feedstock for biobutanol production

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Abstract

Potato peel from a snack factory was assessed as a possible feedstock for biobutanol production. This lignocellulosic biomass was subjected to various physicochemical pretreatments (autohydrolysis, and hydrolysis with dilute acids, alkalis, organic solvents or surfactants) under different conditions of temperature, time and reagent concentrations in order to maximise the release of simple sugars and to minimise the generation of fermentation inhibitors. The pretreated biomass was subsequently subjected to a conventional enzymatic treatment to complete the hydrolysis. Autohydrolysis at 140 ºC and 56 min was the most adequate pretreatment, releasing 37.9 ± 2.99 g/L sugars from an aqueous mixture containing 10% (w/w) potato peel (sugar recovery efficiency $55 \pm 13\%$). The fermentability of the hydrolysates was checked with six strains of *Clostridium beijerinckii*, *C. acetobutylicum*, *C. saccharobutylicum* and *C. saccaroperbutylacetonicum*. The strain *C. saccharobutylicum* DSM 13864 produced 2.1 g/L acetone, 7.6 g/L butanol and 0.6 g/L ethanol in 96 h (0.186 g_B/g_S), whereas the strain *C. saccharoperbutylacetonicum* DSM 2152 generated 1.8 g/L acetone, 8.1 g/L butanol and 1.0 g/L ethanol in 120 h (0.203 g_B/g_S). Detoxification steps of the hydrolysate before fermentation were not necessary. Therefore, potato peel might be an interesting feedstock for biorefineries focused on butanol production.

Keywords

Potato peel; lignocellulosic wastes; pretreatment; ABE fermentation; biorefinery

1. Introduction

The concept of biorefinery appeared during the 1990s as a result of fossil fuel scarcity and the growing trend in the use of biomass as a raw material [1]. The purpose of biorefineries is the generation of biofuels, energy, biomolecules and non-food products from (preferably residual) biomass without competing with other sectors [2]. Among these bioproducts, 1-butanol has awakened interest, due to its numerous industrial applications as solvent, extractant, base-product or biofuel [3,4]. Nowadays, butanol is mainly produced via petrochemical processes, because the traditional industrial acetone-butanol-ethanol (ABE) fermentation employed expensive raw materials, like cereals, potatoes or corn [5]. However, the use of cheap residual lignocellulosic biomass could make industrial biobutanol fermentation profitable again, provided that adequate pretreatments exist to release simple sugars from the complex lignocellulosic network [6,7]. Solventogenic bacteria from the genus *Clostridium* which had been employed in industrial ABE fermentation are not able to directly metabolise the polysaccharides (cellulose and hemicellulose) present in lignocellulosic biomass. Therefore, at present an expensive pretreatment is needed to release simple sugars (mainly glucose and xylose) from the intricate lignocellulosic fibre network [8]. This pretreatment usually consists of a physicochemical treatment to alter lignocellulose and it is followed by the enzymatic hydrolysis of polysaccharides.

The world production of potatoes in 2014 attained 381,682,144 tonnes; of which 60,686,830 tonnes were harvested in the European Union [9]. It is estimated that nearly 14% of the global potato crop is destined to potato processing industries, where potatoes are mainly used for the manufacture of potato chips, frozen French fries or starch [10]. Potatoes are usually peeled during processing, which may be accomplished by steam, abrasive or lye peeling [11]. The average generation of potato peel in the EU-28 between 2010 and 2013 reached about 3,013,000 tonnes/year [12]. Potato peel could be a source of valuable compounds and fibres [11]; in addition, this by-product has been regarded as a potential feedstock to be converted into biofuels [13,14,15].

Some potato wastes and by-products have been evaluated for butanol production, like waste starch [16], potato powder [17] and potato peel with important starch proportions [18]. However, attempts to obtain butanol from lignocellulosic potato peel have been unsatisfactory [19].

In the present work, potato peels from a snack factory were studied as a possible raw material for biobutanol production. This potato by-product contained a high content of lignin (~33%). In the first place, five different soft physicochemical pretreatments (autohydrolysis, acids, alkalis, organic solvents or surfactants) with several reagents were compared, and working parameters (temperature, time and reagent concentration) were optimised. The pretreated biomass was subsequently subjected to a conventional enzymatic treatment to complete the hydrolysis. The main objective of these pretreatments was to maximise the amount of simple sugars released and to minimise the generation of fermentation inhibitors. The fermentability of potato peel hydrolysates was tested with six solventogenic bacterial strains belonging to the species *Clostridium beijerinckii*, *C. acetobutylicum*, *C. saccarobutylicum* and *C. saccharoperbutylacetonicum*.

2. Material and methods

2.1. Chemicals and reagents

Chemical pure grade HNO_3; analytical grade HCl, H_2SO_4, NaOH and KOH; and HPLC grade methanol were supplied by Panreac (Castellar del Vallès, Spain). Analytical grade ammonia solution and ethanol were obtained from Scharlab (Sentmenat, Spain). Tween 80 and HPLC grade acetone were provided by Sigma-Aldrich (Steinheim, Germany). Polyethylene glycol 6000 (PEG 6000) was purchased from Acros Organics (Geel, Belgium), while cetyltrimethylammonium bromide (CTAB) was supplied by Ankom Technologies (Macedon, NY, USA).

The enzymes Celluclast 1.5L and Spirizyme Fuel were kindly supplied by Novozymes (Bagsvaerd, Denmark) and Cellic CTec2 was kindly provided by Novozymes (Tianjin, China). The measured enzymatic activities were 88 FPU/mL for Celluclast 1.5L, 124 FPU/mL for Cellic CTec2 and 877

AGU/mL for Spirizyme Fuel, whereas protein contents were 105 mg/mL for Celluclast 1.5L, 176 mg/mL for Cellic CTec2 and 226 mg/mL for Spirizyme Fuel.

2.2. Biomass description and processing

Fresh potato peel containing 76.94% moisture was kindly provided by Aperitivos Gus S.L. (Riego de la Vega, León, Spain) in summer 2016. This snack factory uses abrasion techniques for the peeling. The raw biomass was dried during a week in the open air and then dried further in an oven at 45 °C until constant weight. The dried biomass was ground in a SM100 Comfort rotary mill (Retsch GmbH, Haan, Germany) and sieved to a size of 0.5-1.0 mm.

Moisture, ash, structural carbohydrates (cellulose and hemicellulose), Klason lignin, proteins, fats and total phenolic compounds were analysed as described by Hijosa-Valsero et al. [20]. Starch was analysed by polarimetry according to Spanish national regulations [21]. The chemical composition of potato peel can be found in Table 1.

Table 1. Chemical composition of dry potato peel.

Components	Amount
Total carbohydrates (%)	43.20
Soluble carbohydrates (%)	0.43
Cellulose (%)	8.3
Hemicellulose (%)	7.41
Starch (%)	23.01
Lignin (%)	32.88
Protein (%)	10.73
Fats (%)	2.45
Ash (%)	7.45
Moisture (%)	5.26
Total phenolic compounds (mg/g)	2.5

2.3. Pretreatment

2.3.1. Selection of chemical compounds for the physicochemical treatment

Preliminary experiments were carried out in order to select the most suitable reagents for potato peel physicochemical pretreatment. The assessed compounds and their concentrations are shown in Table 2. A biomass-to-solvent ratio of 10% was chosen, since the great water-absorption capacity of potato peel hindered the use of higher biomass concentrations. The mixture of potato peel (10%) and aqueous solution (90%) generates a mash which is difficult to shake with laboratory devices.

The pretreatments were carried out in triplicate in an autoclave at 121°C during 2 h as explained elsewhere [20]. Subsequently, an enzymatic hydrolysis was performed with 409 μl Celluclast 1.5L and 100 μl Spirizyme Fuel per 100 g solid/aqueous mixture (see section 2.3.2 for pH and incubation details), and the sample was analysed for sugars and fermentation inhibitors (see section 2.5).

The most appropriate reagent of each chemical group (acids, alkalis, organic solvents and surfactants) was selected to carry out an optimisation of pretreatment conditions as described in section 2.3.2. According to the results, HNO_3, NH_4OH, acetone and PEG 6000 were selected as the most suitable reagents (Table 2).

2.3.2. Optimisation of pretreatment conditions

Pretreatments were performed with a high-pressure 2-L reactor made of alloy Carpenter-20 (Parr Instrument Company, Moline, IL, USA) with a solid-to-solvent ratio of 10% (w/w); using water (autohydrolysis) or aqueous solutions of HNO_3, NH_4OH, acetone or PEG 6000. The reactor operation procedure followed a previously described methodology [20]. For working temperatures and times, see Table 3.

After the physicochemical pretreatment, the solid/liquid mixture coming from the reactor was subjected to an enzymatic hydrolysis at pH 5.0 (citrate buffer 50 mM) and 50 °C during 72 h, by adding 290 μl Cellic CTec2 and 100 μl Spirizyme Fuel per 100 g of initial solid biomass [20]. After enzymatic hydrolysis, the samples were filtered and prepared for chemical analyses as explained in section 2.5. Simple sugars as well as potential fermentation inhibitors were analysed.

For the optimisation of working conditions (independent variables: temperature, time and reagent concentration), complete central design (CCD) and response surface methodology (RSM) experiments were performed for each

pretreatment type. The equations calculated via RSM were used to estimate the optimal values for the three independent variables that would release the highest amount of total sugars and the lowest amount of total inhibitors in the broth after the physicochemical treatment in the reactor and the subsequent enzymatic hydrolysis. Then, all the estimated optimal points were validated experimentally.

Table 2. Reagents selected for each AFW in the preliminary autoclave tests. *Notes*: *Reagent percentages are expressed in w/w. **Superscripts (a, b) represent statistical differences among treatments (p < 0.05) calculated with Tukey's HSD test. For a certain treatment type (e.g. acids), two reagents with distinct letters differ significantly. ***Total inhibitors were calculated as the sum of formic acid, acetic acid, levulinic acid, furfural, 5-hydroxymethylfurfural (5-HMF) and total phenolic compounds concentrations. ****Methanol offered better results than acetone and ethanol, but finally acetone was chosen due to its lower price and toxicity.

Treatment type	Reagent*	Total sugars (g/L)**	Total inhibitors (g/L)***	Selected reagent
Autohydrolysis	-	-	-	-
Acid	2% H_2SO_4	30.7 ± 2.15 [a] **	2.73 ± 0.23 [ab]	HNO_3
	2% HCl	29.6 ± 0.95 [a]	3.20 ± 0.26 [b]	
	2% HNO_3	41.2 ± 1.11 [b]	2.45 ± 0.21 [a]	
Alkali	2% NaOH	30.1 ± 5.44 [a]	6.16 ± 1.85 [a]	NH_4OH
	2% KOH	35.0 ± 1.34 [ab]	4.65 ± 0.05 [ab]	
	2.5% NH_4OH	43.2 ± 0.49 [b]	2.11 ± 0.03 [b]	
Organic solvent	40% Ethanol	34.6 ± 0.70 [a]	0.74 ± 0.03 [a]	Acetone****
	40% Methanol	37.0 ± 0.60 [b]	0.88 ± 0.02 [b]	
	40% Acetone	35.5 ± 0.10 [a]	0.96 ± 0.03 [c]	
Surfactant	3% Tween 80	43.7 ± 2.13 [a]	4.65 ± 0.18 [a]	PEG 6000
	3% PEG 6000	38.6 ± 0.98 [b]	0.87 ± 0.06 [b]	
	3% CTAB	38.3 ± 2.43 [b]	1.00 ± 0.03 [b]	

Table 3. General characteristics of the complete central design (CCD) experiments performed to optimize the physicochemical pretreatment of potato peel in the reactor. *Note*: Reagent concentrations refer to the pure substance.

	Factors (variables to optimize)	Runs	Number of cube, central and axial points	Alpha	Axial ranges
Autohydrolysis	2	13	4, 5, 4	1.41421	T (ºC): 120-220 t (min): 5-120
Acids	3	20	8, 6, 6	1.68179	T (ºC): 100-200 t (min): 5-120 Acid (%, w/w): 0.5-3.5
Alkalis	3	20	8, 6, 6	1.68179	T (ºC): 60-200 t (min): 5-120 Alkali (%, w/w): 0.5-4.5
Solvents	3	20	8, 6, 6	1.68179	T (ºC): 100-200 t (min): 5-120 Solvent (%, w/w): 10-70
Surfactants	3	20	8, 6, 6	1.68179	T (ºC): 60-200 t (min): 5-120 Surfactant (%, w/w): 0.3-5.7

2.4. Fermentation of liquid hydrolysates

Various solventogenic strains were assessed for the fermentation of potato peel hydrolysates. The strain *Clostridium beijerinckii* CECT 508 was obtained from the Spanish Collection of Type Cultures (CECT, Paterna, Spain), whereas the strains *C. acetobutylicum* DSM 1732, DSM 1733 and DSM 1738, *C. saccharobutylicum* DSM 13864 and *C. saccharoperbutylacetonicum* DSM 2152 were purchased from the German Collection of

Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany).

Strain culture for *C. beijerinckii* CECT 508 was performed according to Díez-Antolínez et al. [22] until a liquid inoculum containing an approximate bacterial density of $5 \cdot 10^8$ cells/mL was obtained. For all the other strains, lyophilised cells were resuspended in 10 mL sterile medium containing 19 g/L Reinforced Clostridial Medium - RCM (Oxoid, Basingstoke, UK) and 10 g/L lactose. This medium was incubated during 24 h at 35 °C under anaerobic conditions. Then, 1.5 mL were transferred to a sterile cryogenic vial and 0.4 mL glycerol (80% v/v) were added. The vials were closed, shaken and stored at -80°C until being used. For cellular reactivation, a loopful of the thawed glycerinate was spread on a Petri dish containing 38 g/L RCM and 20 g/L agar, and the dish was incubated at 35 °C under anaerobic conditions until colonies were visible (1-3 mm). Then, a colony was transferred to 50 mL of sterile liquid medium (19 g/L RCM, 10 g/L glucose). Afterwards, gaseous N_2 was injected into the headspace of the closed bottles during 5 min to obtain anaerobic conditions. The bottles were incubated for 24 h at 35 °C and were employed as inocula, containing an approximate bacterial density of $5 \cdot 10^8$ cells/mL.

For fermentability tests, hydrolysate samples were filtered through a nylon mesh (30 denier) and the filtrate was centrifuged at 2480 x g during 10 min (centrifuge Jouan CR3i, Château-Gontier, France). Afterwards, hydrolysates were supplemented with 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 $MgSO_4 \cdot 7H_2O$, 0.5 g/L cysteine and 5 g/L $CaCO_3$, autoclaved and adjusted to pH 6.0 [20]. Then, 1.5 mL of the corresponding inoculum were added to 48.5 mL of fermentation medium in rubber-capped bottles, where gaseous N_2 was bubbled during 5 min to guarantee anaerobic conditions. Fermentation bottles were incubated at 35 °C and 100 rpm in an Infors HT Minitron orbital shaker (Infors AG, Bottmingen, Switzerland) during 72-144 h. Fermentation controls consisted of aqueous solutions containing glucose at similar concentrations to those of total simple sugars in potato peel hydrolysates, and supplemented with the abovementioned nutrients. All experiments were performed in triplicate.

2.5. Chemical analyses of hydrolysates and fermented broths

Aqueous samples of hydrolysates and fermented broths were centrifuged at 12,000 x g in a microcentrifuge for 3 min (Minispin, Eppendorf, Hamburg, Germany). The supernatant was filtered through a nylon syringe filter (0.20 μm pore; Agilent, Santa Clara, CA, USA) prior to analysis. The sugars cellobiose, glucose, xylose, rhamnose and arabinose, and the potential inhibitors formic acid, acetic acid, levulinic acid, 5-hydroxymethylfurfural (5-HMF) and furfural were analysed by HPLC-RID as described by Hijosa-Valsero et al. [20]. Other inhibitors, like phenolic compounds, were analysed by Folin-Denis' assay. Total phenolic compounds were expressed as gallic acid equivalents (GAE). Fermentation metabolites, like acetone, butanol, ethanol, acetic acid and butyric acid were determined by GC-FID according to Hijosa-Valsero et al. [20].

Fermentation yields ($Y_{i/S}$, g/g) were defined as the ratio between the metabolite (i) produced and the total sugars consumed (S). Metabolite productivity rates (W_i, g/(L·h)) were obtained as the ratio between the metabolite (i) expressed in concentration (g/L) and the fermentation time (h). Sugar recovery or sugar conversion efficiency (%) was calculated as the ratio between the mass of simple sugars in the hydrolysate and the total mass of carbohydrates in the untreated potato peel.

2.6. Statistical analyses

One-way ANOVA and Tukey's HSD test were applied to assess comparisons among treatments using the software Statistica 7 (StatSoft Inc., Tulsa, OK, USA); differences were considered significant when $p < 0.05$. For the optimisation step, Response Surface Methodology (RSM) designs were generated and interpreted with the software Minitab 16 (Minitab Inc., State College, PA, USA).

3. Results and discussion

3.1. Pretreatment of potato peel

Optimal working conditions in the reactor (temperature, time and amount of reagent) were calculated via RSM experimental design for

autohydrolysis, nitric acid, ammonium hydroxide, acetone and PEG 6000 pretreatments. Table 4 shows calculated optimal values for each parameter and pretreatment. The lowest operational temperature was obtained for acid hydrolysis (110 ºC), whereas the highest one was recorded for surfactant-mediated hydrolysis (146 ºC). Treatment times were relatively short (always below 90 min), especially for PEG 6000 (Table 4). The amount of reagents needed to perform an efficient hydrolysis was between 0.5 and 10% (Table 3, Table 4). These values are in agreement with working parameters reported in literature for the pretreatment of other lignocellulosic wastes, or are even lower. For instance, autohydrolysis is usually performed at 170-190 ºC during 5-60 min [23,24,25]. For other treatment types, the most common reagents used are 0.1-3% H_2SO_4 for acid pretreatments [7]; 2.5-20% NH_4OH or 0.5-2% NaOH for alkaline pretreatments [26,27]; 30-80% ethanol or acetone for organic solvent pretreatments [28,29]; and 0.2-0.4% Tween and PEG 6000 for surfactant pretreatments [30,31]. These pretreatments employing reagents are generally carried out at 120-200 ºC during 5-100 min [28,30,32,33].

Theoretical values estimated for total sugars and total inhibitors were validated experimentally (Table 4). Some small differences were observed between estimated and experimental values (especially in the case of nitric acid), but in general the models were acceptable. The most efficient pretreatment for the release of simple sugars was nitric acid hydrolysis (43.7 g/L total sugars), followed by PEG 6000 hydrolysis (39.0 g/L total sugars) (Figure 1a). The rest of the pretreatments were able to release total sugar concentrations of 34.9-37.9 g/L. These results are lower than those reported by Khawla et al. [34] for the enzymatic hydrolysis of potato peel by *Bacillus* sp. enzymes during 24 h, where a broth with 69 g/L total sugars from a sample containing 15% potato peel was obtained. However, the starch amount in potato peel in that work was twice greater than in the present study (48.46% starch [34]). Similarly,

Abd-Alla et al. [18] worked with potato peel composed of 68.3% total sugars and 18.5% protein (and presumably insignificant amounts of lignin), and obtained a broth containing 46 g/L sugars from a potato peel suspension (67.4 g/L solids) subjected to autoclaving and without the need of enzymatic hydrolysis. Considering the total carbohydrate composition of potato peel (43.20%; Table 1) and the hydrolysate volumes collected, sugar recovery efficiencies were 55 ± 13% for autohydrolysis, 67 ± 9% for nitric acid, 56 ± 2% for ammonium, 47 ± 2 % for acetone and 57 ± 2% for PEG 6000. Data about sugar recovery efficiencies after potato peel pretreatment are scarce. Arifin et al. [19] reported a sugar overall recovery of 10% after autoclaving (i.e. autohydrolysis) and performing an enzymatic hydrolysis on potato peel.

Regarding inhibitors, nitric acid pretreatment generated the highest concentration of these compounds, especially for formic acid (0.58 g/L), which can be an important disadvantage for bacterial fermentation (Figure 1b). On the other hand, the hydrolysis mediated by PEG 6000 generated less inhibitors than any other pretreatment (Table 4). It must be noted that the production of some inhibitors, like levulinic acid, furfural and 5-hydroxymethylfurfural was extremely low for all the tested pretreatments (Figure 1b), and their concentrations were below quantification limits (0.05 g/L) in most cases. Furfural is a degradation product from pentoses and, given the low concentrations of xylose and arabinose in the sample (Figure 1a), the practical absence of furfural is expectable. It has been reported that concentrations above 0.24-0.5 g/L formic acid [4,35], 5 g/L acetic acid [36], 2.9 g/L furfural [4], 3 g/L 5-HMF [37] and 1 g/L phenolic compounds [38] can be detrimental to solventogenic Clostridia. Consequently, taking into account only the analysed inhibitors and their concentrations, potato peel hydrolysates obtained by autohydrolysis, ammonium, acetone and PEG 6000 treatments could be potentially fermentable.

Table 4. Optimal working conditions, estimated responses (calculated with RSM) and validation experimental responses for potato peel in the high-pressure reactor for each pretreatment method. *Notes*: (a) These concentrations were measured after subjecting potato peel to the physicochemical pretreatment and a subsequent enzymatic hydrolysis. (b) Reagent concentrations refer to the pure substance.

|	Physicochemical treatment optimal conditions (RSM)			Estimated responses (RSM)[a]		Experimental responses[a]	
	T (°C)	t (min)	Reagent (%, w/w)[b]	Total sugars (g/L)	Total inhibitors (g/L)	Total sugars (g/L)	Total inhibitors (g/L)
Autohydrolysis	140.2	56.1	-	36.6	1.43	37.9 ± 2.99	1.41 ± 0.08
Acid (HNO₃)	109.6	83.4	1.81	39.0	2.00	43.7 ± 0.37	2.02 ± 0.24
Alkali (NH₄OH)	111.8	29.0	0.50	38.5	1.40	36.9 ± 0.55	1.59 ± 0.03
Solvent (acetone)	127.7	85.1	10.0	34.0	1.50	34.9 ± 2.74	1.43 ± 0.05
Surfactant (PEG 6000)	145.6	5.0	1.92	41.0	1.00	39.0 ± 1.00	1.06 ± 0.06
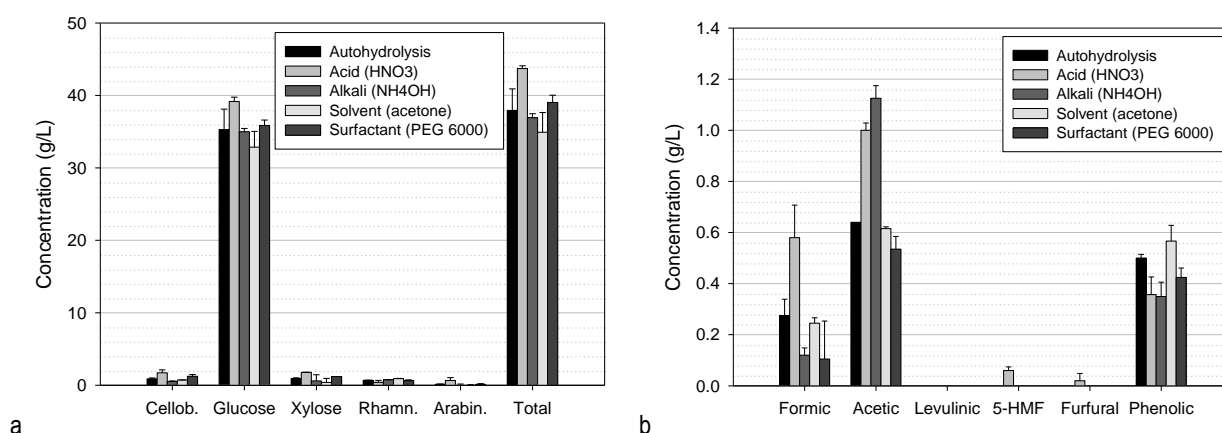
Figure 1. Composition of potato peel hydrolysates for each pretreatment under optimal conditions. a) Concentration of released sugars. b) Concentration of inhibitors generated. *Note*: Concentrations were measured after subjecting potato peel to the physicochemical pretreatment and a subsequent enzymatic hydrolysis.

3.2. Hydrolysate fermentability and strain selection for butanol production

All the hydrolysates obtained with the reactor pretreatment and the subsequent enzymatic hydrolysis (see conditions in Table 4) were supplemented with nutrients and fermented with *C. beijerinckii* CECT 508 as described in section 2.4. Only autohydrolysis and PEG 6000 pretreatments produced fermentable hydrolysates (Figure 2), attaining butanol concentrations of 3.36 ± 1.90 and 2.76 ± 2.28 g/L, respectively; slightly below the control result, which was 5.03 ± 1.82 g/L (Figure 2). The hydrolysate obtained by acetone pretreatment contained remains of the added acetone, which explains the presence of this solvent in the fermented broth. In any case, the low butanol concentration obtained in the control

solution by *C. beijerinckii* CECT 508 indicates that this strain might not be the most suitable for the fermentation of a broth containing 35-39 g/L total sugars, mostly glucose (Figure 1a).

Therefore, the six solventogenic strains listed in section 2.4 were evaluated and compared in order to check their ability to produce butanol from potato peel hydrolysates. In this case, the selected pretreatment was autohydrolysis, because its hydrolysate seemed the most easily fermentable by *C. beijerinckii* CECT 508 in the previous experiment (Figure 2); a fact which could be related to its relatively low inhibitor content (Figure 1b) and to the lack of added reagents, like nitrate from HNO₃ or NH₄⁺ from ammonium hydroxyde, which could imbalance fermentation. The choice of autohydrolysis is in agreement with

the results reported by Ben Taher et al. [39], who evaluated potato peel as a feedstock for ethanol production. They compared autohydrolysis, dilute acid and dilute alkali pretreatments (121 °C, 30 min, 10% biomass-to-solvent ratio), followed by enzymatic hydrolysis, and concluded that autohydrolysis produced the best results. However, the composition of the potato peel treated by Ben Taher et al. [39] was 33.5% cellulose, 5.5% hemicellulose, 42% starch and 4.7% lignin, which makes it remarkably different from the biomass of the present work (Table 1), which contained much more lignin (33%) and less starch and cellulose (23% and 8%, respectively).

As shown in Figure 3, the strains *C. saccharobutylicum* DSM 13864 and *C. saccharoperbutylacetonicum* DSM 2152 performed successfully and were able to produce butanol concentrations above 7 g/L and to practically deplete sugars, both in control solutions and real potato peel hydrolysates. On the contrary, the other strains produced relatively low butanol concentrations (below 5 g/L), even in the case of control solutions; which could indicate that these strains are not the most suitable for the fermentation of a broth composed of approximately 40 g/L glucose. Butanol production by *C. beijerinckii* CECT 508 from potato peel hydrolysate was poor, probably because it cannot cope with that sugar mixture in an industrially-efficient way (Figure 3, Table 5). The tested strains of *C. acetobutylicum* (DSM 1732, DSM 1733 and DSM 1738) did not seem to be adapted to potato peel hydrolysate fermentation, since they produced butanol concentrations below 0.6 g/L with yields lower than 0.023 g/g (Table 5). In the case of the successful fermentations of potato peel hydrolysate by *C. saccharobutylicum* and *C. saccharoperbutylacetonicum*, the time needed to reach the maximum butanol concentration was 96 and 120 h, respectively (Table 5). Butanol yields were 0.186 g/g and 0.203 g/g for these strains, and it must be highlighted that sugar consumption was almost total in both cases, which is an indicator of an efficient fermentative process. Butanol productivity values (W_B) were below 0.08 g/(L·h) for all the strains (Table 5). Normally, *C. saccharobutylicum* and *C. saccharoperbutylacetonicum* strains were employed in industrial fermentations of molasses, obtaining ABE yields of 0.27-0.33 g/g and 0.27-

0.34 g/g, respectively; although these yields halved when using other substrates like corn mash [40]. These strains have also been reported to be able to ferment agriculture and agro-food wastes. For instance, *C. saccharobutylicum* NCP P262 (=DSM 13864) produced butanol from white grape pomace [41], or from *Panicum virgatum* and *Phragmites australis* hydrolysates [42], whereas *C. saccharoperbutylacetonicum* ATCC 27022 (=DSM 2152) was able to ferment bagasse and rice straw hydrolysates after detoxification [43].

It is known that the performance of solventogenic strains varies depending on the substrate nature. In fact, Green [44] indicated that industrial strains are selected based on performance against specific substrates and developed for growth and fermentation on specific feedstocks. Shaheen et al. [45] carried out a comparative study with several industrial *Clostridium* strains, which were fed with laboratory fermentation medium, molasses or corn. They observed that the species *C. acetobutylicum* had the greatest potential for starch-based substrates, *C. saccharobutylicum* appeared to have potential for use in mixed agricultural and waste-based substrates, *C. saccharoperbutylacetonicum* possessed versatile strains that could be useful on mixed substrates, whereas *C. beijerinckii* did not perform particularly well in any of the three fermentation substrates used in that study. In particular, Shaheen et al. [45] referred to *C. beijerinckii* NCIMB 8052 strain (=CECT 508), and hypothesized that its low performance could be due to the application of culture conditions unsuitable for this strain or to the loss of its solvent-producing ability. Actually, *C. beijerinckii* CECT 508 has been successfully employed for cheese whey [22] or apple pomace fermentation [20]; but in these cases the strain had been previously subjected to various sporulation cycles.

In the past years, starch-rich potato media have been successfully fermented yielding high butanol concentrations. For instance, Kheyrandish et al. [16] fermented aqueous solutions containing waste potato starch (60 g/L) with *C. acetobutylicum* NRRL B-591 and obtained 9.9 g/L butanol in batch reactors with free cells and 15.3 g/L butanol with cells immobilised in alginate-borate beads. They reported that maximum butanol yields (0.21 g/g) were obtained for an

initial starch concentration of 20 g/L. Grobben et al. [17] performed a fermentation coupled to an *in situ* perstraction system for solvents recovery, using a solution of 140 g/L potato powder with *C. acetobutylicum* DSM 1731; and obtained 20 g/L ABE solvents and a yield of 0.23 g/g ABE. Abd-Alla et al. [18] prepared a broth from starch-rich potato peel without nutrient supplementation and obtained 10.98 g/L butanol in 8 days with *C. beijerinckii* ASU10. They also observed significant differences among various bacterial species in terms of butanol production [18]. Nevertheless, according to scientific literature, ABE fermentation of lignocellulosic potato peel has not been so successful. Arifin et al. [19] pretreated potato peel by autoclaving the biomass and subjecting it to an enzymatic hydrolysis with the fungus *Aspergillus niger* during seven days, thus obtaining a hydrolysate with 20 g/L sugars; which was converted by *C. acetobutylicum* ATCC 824 into 0.9 g/L butanol with a yield of 0.371 g/g. Accordingly, the present work means a remarkable advance in biobutanol production from lignocellulosic wastes like potato peel, not only from the point of view of generating a hydrolysate with low inhibitor

concentrations that does not require a detoxification step, but also in terms of butanol concentrations.
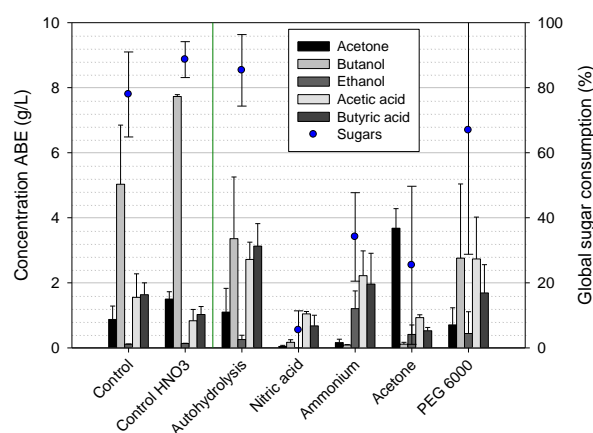

Figure 2. Fermentation metabolites and sugar consumption of *C. beijerinckii* CECT 508 for potato peel hydrolysates obtained with different pretreatments (fermentation time 96 h). *Note*: The general control contained 36 g/L glucose, whereas the nitric acid control contained 41 g/L.
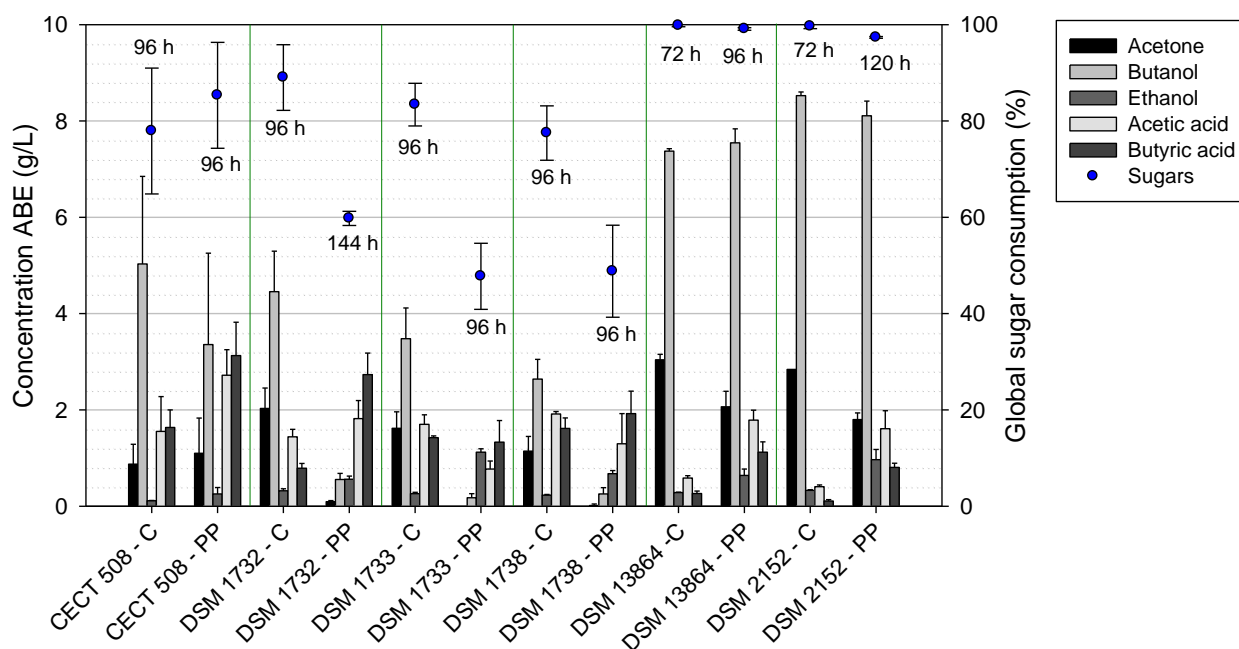

Figure 3. Fermentation metabolites and sugar consumption of various solventogenic strains feeding on potato peel hydrolysates obtained by autohydrolysis. The times indicated above each group of bars refer to the time needed to complete the fermentation. *Notes*: Initial sugar concentrations in the potato-peel hydrolysate (PP): 1.03 g/L cellobiose, 38.14 g/L glucose, 0.93 g/L xylose, 0.66 g/L rhamnose and 0.17 g/L arabinose. Controls (C) consisted of glucose solutions at a similar concentration.

Table 5. Efficiency parameters for butanol production from autohydrolysis-pretreated potato peel by different solventogenic strains.

Strain	Fermentation time (h)	Sugar consumption (%)	Butanol,(g/L)	Yield,$Y_{B/S}$ (g/g)	Productivity,W_B (g/L·h)
Clostridium beijerinckii CECT 508	96	85.3±11.0	3.36±1.90	0.100±0.045	0.023±0.013
Clostridium acetobutylicum DSM 1732	144	59.8±1.5	0.55±0.13	0.023±0.006	0.004±0.001
Clostridium acetobutylicum DSM 1733	96	47.7±6.9	0.18±0.09	0.009±0.003	0.002±0.001
Clostridium acetobutylicum DSM 1738	96	48.8±9.6	0.26±0.13	0.012±0.004	0.003±0.001
C. saccharobutylicum DSM 13864	96	99.1±0.3	7.55±0.29	0.186±0.007	0.079±0.003
C. saccharoperbutylacetonicum DSM 2152	120	97.3±0.2	8.11±0.31	0.203±0.008	0.068±0.003

3.3. Perspectives of potato peel utilization as a feedstock for ABE fermentation

One of the advantages of potato peel as a raw material for biorefineries is its continual production. Different varieties of potatoes are grown throughout the year to satisfy market demands and, in addition, surplus potatoes can be stored during weeks or months. However, the chemical composition of potato peel could vary depending on the cultivar variety and on the harvest season; which implies that operation conditions at biorefineries should be adaptable enough to deal with potential changes in biomass composition. In addition, the great capacity of potato peel to absorb water implies that the industrial equipment should be designed in such a way that agitation devices in pretreatment reactors are able to move mashes with a low fluidity. It must be remembered that in the present study a limit of 10% biomass-to-solvent ratio was established in order to avoid the blockage of the pretreatment reactor rotor.

Although the fermentation of potato peel hydrolysates seems successful for biobutanol production, there are still some technical issues which need to be improved before the process can be implemented at industrial scale. In the first place, the enzymatic hydrolysis step needs further enhancement to reduce treatment times, enzyme doses and increase the stability and catalytic activity of enzymes [46]. The production of ligninolytic or cellulolytic enzymes in the own biorefinery facilities could reduce reagent costs. Other alternatives like simultaneous saccharification and fermentation could improve the economic performance of the process, both with cellulolytic enzymes and solventogenic *Clostridium* strains or with a coculture of cellulolytic and solventogenic species [8], or the design of genetically-engineered microorganisms that can directly convert polysaccharides into butanol [47].

Another important matter hindering the implementation of butanol biorefineries is the technical limitation to cost-efficiently separate and purify acetone, butanol and ethanol from the fermentation broth, which was traditionally made by distillation. Different methods like gas stripping, pervaporation, perstraction or adsorption are being assessed to improve the energetic efficiency of this process [48].

Butanol-producing strains require the addition of several nutrients and micronutrients to the fermentation broth (yeast extract, nitrogen, iron salts, etc.), which increases production costs. Moreover, nutritional needs vary depending on the strain and on the hydrolysate composition; therefore, in order to save reagents and ameliorate butanol production, it is extremely recommendable to optimise nutrient composition and concentrations for each particular case. Furthermore, the use of alternative nutrient sources (like wastes and by-products from other processes) could reduce fermentation costs and even increase solvent production [49].

Potato peel can be used for ABE production after an autohydrolysis and an enzymatic hydrolysis, without the need of a detoxification step prior to fermentation. This constitutes a double advantage. In the first place, a physicochemical pretreatment like autohydrolysis avoids the use of any reagent

except water, which reduces economic and environmental costs. Secondly, the possibility of directly fermenting the hydrolysate without removing inhibitors by adsorption columns or similar devices (i.e. detoxification), simplifies infrastructures and operation costs.

In the present study, total sugar concentrations in potato peel hydrolysate were about 40 g/L. This concentration was sufficient for the correct ABE fermentation of this broth by certain bacterial strains. This sugar value, low as it may seem, has a positive side: the release of higher sugar concentrations, usually by more severe pretreatments, implies the generation of greater concentrations of inhibitors. Hence, by finding an equilibrium point between sugar concentrations and inhibitors generation during the physicochemical pretreatment, it is possible to obtain a readily fermentable broth. As a consequence, the paradigm of measuring a pretreatment's efficiency only by the sugar concentration in its hydrolysate might not be the most appropriate.

4. Conclusions

Potato peel from a snack factory proved to be a suitable feedstock for biobutanol production. The pretreatment of this lignocellulosic biomass was a relatively easy process, involving potato peel autohydrolysis at a 10% biomass-to-solvent ratio and a subsequent enzymatic hydrolysis. From the six bacterial strains used, two of them were able to obtain promising butanol concentrations, which underlines the importance of strain selection for the fermentation of complex samples. The hydrolysate was directly fermentable without the need of a detoxification step, thanks to a statistical optimisation process to maximise sugar release and minimise inhibitor generation during the pretreatment. Further research is needed before the use of agro-food wastes as feedstocks for biobutanol production is economically feasible at industrial scale.

Acknowledgements

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