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Butanol Production by Fermentation of Fruit Residues

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The feedstock of the Acetone-Butanol-Ethanol (ABE) fermentation is a key issue for the economic success of the biotechnological route to produce biobutanol. Residues from agro-alimentary industries are particularly interesting as renewable substrates for the ABE fermentation because they are abundant and un-competitive with food sources. The residues are also a pressing issue for industries because more than 50% of the processed feedstocks are discharged and their disposal is particularly expensive. However, the high fraction of sugars of the residues makes them a promising interesting feedstock for the production of butanol.
This contribution is about the characterization of the ABE fermentation by *Clostridium acetobutylicum* DSM 792 using sugars from fruit peels. Apple and pear peel extracts were tested as substrate for the fermentation. Batch tests were carried out under a wide interval of peels to water mass ratio. The conversion process was characterized in terms of metabolites and cell production, sugars conversion, specific rate of butanol production and of sugar consumption, butanol and cell yields.
The fermentation tests with feedstock peels to water mass ratios lower than 1/6 were characterized by total sugar conversion and low butanol concentration (<8.5 g/L). Tests with feedstock peels to water ratios higher than 1/8 were characterized by high butanol production (about 14 g/L) and incomplete sugar conversion.

1. Introduction

The increase in prices of petroleum based fuels, future depletion of worldwide petroleum reserves and environmental policies to reduce CO_2 emissions have stimulated research into the development of biotechnology to produce chemicals and fuels from renewable resources (Parekh et al., 1999). The most commonly used biofuels are ethanol and butanol. Butanol, is produced biologically from renewable biomass by *Clostridium* spp. under strictly anaerobic condition (Jones and Woods, 1986), together with acetone, and ethanol. Acetone, butanol and ethanol (ABE) are: common solvents in many industries, building blocks for chemicals, and have a high potential for replacing petrochemical derived energy vectors.
Substrate costs can make up to about 63% of the total cost of ABE production (Raganati et al., 2015a). However, the ability of saccharolytic clostridia to utilize a wide spectrum of carbohydrates open the possibility to use of alternative cheaper substrates (Jones and Woods, 1986). Indeed, the availability of an abundant supply of low-cost substrate is essential in making the ABE process economically viable (Jones and Woods, 1986), Hence, substrates such as agricultural residues, including wheat straw, barley straw, maize stover, wood hydrolysate, and switchgrass as well as dairy industry wastes are potential feedstock alternatives because are abundant and for free (Qureshi et al., 2010).
Agro-industrial residues - such as peels, seeds, and pulps - are about 50% of raw processed fruits. These residues are not characterized by any commercial interest and are traditionally incinerated with other combustible municipal wastes for generation of heat and energy. It should be realized that agro-industrial residues indeed contain high level of moisture and this may lead to the production of dioxins during their combustion together with other wastes of low humidity and high calorific value (Katami et al., 2004). In addition, the incineration of agro-industrial residues contributes to the increase of the CO2 concentration in the atmosphere. These issues suggest that an appropriate management of agro-industrial residues is strongly required (Mamma et al., 2008).

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Agro-industrial residues are mainly composed of carbohydrate polymers (starch, cellulose and hemicelluloses), lignin, proteins, lipids, organic acids, and a small fraction of ash (Uçkun et al., 2014). Hydrolysis of carbohydrate of agro-industrial residues may result in the breakage of glycoside bonds with releasing polysaccharides as oligosaccharides and monosaccharides, which are more amenable to fermentation (Oberoi et al., 2010). At the same time this hydrolysis process can release various inhibitors on the growth and ABE production by clostridia such as ferulic and p-coumaric acids, HMF, furfural etc.
Total sugar and protein contents in agro-industrial residues are in the range of .35.5–69% and 3.9–21.9%, respectively. Therefore, agro-industrial residues have been used as the sole microbial feedstock for the development of various kinds of value-added bioproducts, including methane, hydrogen, ethanol, butanol, enzymes, organic acids, and biopolymers (Han and Shin,2004). Fuel applications ($200–400/ton biomass) are usually creating more value compared to generating electricity ($60–150/ton biomass) and animal feed ($70–200/ton biomass) (Uçkun et al., 2014).
Among the agro-industrial residues, the fruit peels are a remarkable source of sugars that makes them an interesting candidate for the production of value added products (Sánchez-Orozco et al., 2012; Mtui, 2009) such as butanol. The edible pulp makes up 33 to 85% of the fresh fruit, while the peel and the kernel comprise 7 to 24% and 9 to 40%, respectively. To the authors' knowledge no work on the use of this kind of substrate for butanol production is reported in the literature.
This contribution reports the characterization of the ABE fermentation by *C. acetobutylicum* DSM 792 using sugars from fruit peels. Apple and pear peel extracts were tested as substrate. Batch tests were carried out under a wide interval of peels to water mass ratio.

2. Materials and Methods

2.1 Microorganism

Clostridium acetobutylicum DSM 792 was supplied by DSMZ. Stock cultures were reactivated according to the DSMZ procedure. Reactivated cultures were stored at -80°C. The thawed cells were inoculated into 12 mL synthetic medium containing glucose (30 g/L) and Yeast Extract (YE) (5 g/L) in 15 mL Hungate tubes (pre-cultures). Cells were grown under anaerobic conditions for 48 h at 37 °C, then they were transferred into fermentation bottles (Raganati et al., 2015b).

2.2 Medium

Apples (Annurca type) and pears (Williams type) were from a local store in bulk. The fresh fruits were washed and splitted into pulp and peels. The peel was removed using a sharp knife, and the underlying pulp was removed by gently scraping with its blunt edge. The peels were cut into 1-2 cm pieces then washed thoroughly with deionized water to remove physically adsorbed contamination. The peel pieces were air dried for few days in sunlight then completely dried in oven (60 °C, 2 days) according to procedure reported by Kandari and Gupta (2012) for tests aimed at the bioconversion of vegetable and fruits peels into commercially viable products (Drying procedure was performed for storage reasons). The dried peels were diluted with distilled water and boiled for 30 min before extraction. The peels to water mass ratio was varied in a wide range in order to obtain different sugar concentration; in particular the peels to water mass ratio was set to: 1/6, 1/8, 1/10, 1/12. The boiled biomass was centrifuged (5000 rpm for 30 minutes) and the supernatant rich in sugars (glucose, fructose, and sucrose) was supplemented with 5 g/L of YE, 2 g/L of NH_4Cl, 5 g/L of $CaCO_3$ (final concentrations) and P_2 stock solution (Qureshi and Blaschek, 1999). Table 1 reports the composition of sugars for the different apple peels to water ratio (pear peels extracts had similar sugar composition, within a difference of ± 5%). The fermentation tests with apple and pear peels gave similar results, for this reason in the following sections only the apple peels extract results will be reported.

Table 1: Sugar composition of the apple peels extract as a function of the peels to water mass ratio

Apple peels to water mass ratio	Glucose g/L	Fructose g/L	Sucrose g/L
1/6	41	36	30
1/8	31	27	23
1/10	25	22	18
1/12	20.5	18	15

2.3 Batch fermentation

Pyrex screw capped bottles (100 mL) containing 75 mL medium were used as fermenters. All experiments were carried out in fermenters at rest, at 37 °C, without pH control. The medium was inoculated with 6.25 % (v/v) suspension of active growing pre-cultures. 3 mL of cultures were sampled periodically for cell/metabolites and sugars characterization.

2.4 Analytical procedures

pH was measured off-line in 1.5 mL samples by a pH-meter (Hanna Instruments). Analysis of culture samples was carried out after centrifugation at 10,000 rpm for 10 min. The liquid phase was characterized in terms of sugar and metabolite concentrations. Cell density was determined by measuring the absorbance at 600 nm (Cary-Varian mod. 50 scan UV-VIS spectrophotometer). Calibration tests indicated that the optical density is proportional to *C. acetobutylicum* dry mass under the operating conditions tested, in particular 1 OD_{600} corresponded to 0.4 g_{DM}/L. Sugar concentration was determined by high performance liquid chromatography (HPLC) using an Agilent 1100 system (Palo Alto, CA). The sugars were separated on a 8 μm Hi-Plex H, 30 cm 7.7 mm at room temperature and detected with a refractive index detector. Deionized water was used as mobile phase at a flow rate of 0.6 mL/min. A GC apparatus equipped with a FID, and outfitted with a capillary column poraplot Q (25 m x 0.32 mm) was used. Internal standard (hexanoic acid) was adopted to assess acids and alcohols and their concentrations. ABE, butanol and cell yield ($Y_{ABE/S}$, $Y_{B/S}$ and $Y_{X/S}$) were calculated as mass of ABE, butanol and dry cells produced per mass unit of sugar converted.

3. Results and discussion

Tables 2 reports the results of the batch fermentation tests carried out using apple peels extracts as carbon source. The tests carried out inoculating *C. acetobutylicum* into the fruit peels extracts without any nutrient supplementation (data not reported) pointed out that the microorganism did not grow because of the lack of some indispensable nutrients in the fermentation broth. 1/6, 1/8, 1/10 and 1/12 refer to tests carried out inoculating *C. acetobutylicum* into the apple and pear peels extracts supplemented as described in the "Materials and Methods" section.

Table 2: Relevant data of C. acetobutylicum fermentation tests for the different apple peels to water mass ratios.

Apple peels to water ratio	$Y_{B/S}$ g/g	$Y_{ABE/S}$ g/g	$Y_{X/S}$ g/g
1/6	0.20	0.28	0.05
1/8	0.18	0.23	0.04
1/10	0.13	0.19	0.05
1/12	0.13	0.19	0.06

Figure 1 reports the time-resolved profiles of the concentration of *C. acetobutylicum* cells, sugars, (glucose, fructose, and sucrose), and metabolites (acetic acid, butyric acid, acetone, butanol, and ethanol) as well as of pH, measured during a batch culture. The data in the figure referred to a test carried out with the apple peels extract at peels to water mass ratio set to 1/6.

The initial concentration of glucose-fructose-sucrose was 41-36-30 g/L respectively (see Table 1). The analysis of the data confirmed the typical two-phase behaviour of the fermentation (Jones and Woods 1986): acidogenic phase and solventogenic phase. After a lag phase of about 15 h, the acidogenic phase was characterized by:

i) the continuous conversion of sugars. In particular it can be observed that during the acidogenesis only glucose and fructose were metabolized while sucrose was unconverted;

ii) the increase in the concentration of cells and acids;

iii) the decrease of the pH of the medium.

As the pH approached 4.5 (t_A = 50 h), the solventogenic phase started. This phase was characterized by:

i) the gradual decrease in sugar concentration up to a constant value. It can be observed that the glucose was completely converted after about 50 h while fructose and sucrose was just partially converted (total conversion of sugars of about 66%);

ii) the gradual reduction of cell concentration as a result of cell lysis (Ezeji et al., 2007);

iii) the steady increase in solvent concentration up to a constant value (about 14 g/L of butanol);

iv) the gradual decrease in acid concentration as a result of their reassimilation.

Main results of the batch tests carried out with apple peels extract characterized by peel to water ratios 1/8, 1/10 and 1/12 are reported in Table 2. The comparison with the test carried out at peels to water mass ratio set at 1/6 points out that the initial concentration of glucose-fructose-sucrose is progressively lower as the ratio decreased (Table 1).
The time-resolved profiles of pH, cell concentration, sugar (glucose, fructose and sucrose) concentration, and metabolite (acetic acid, butyric acid, acetone, ethanol and butanol) concentration measured during the batch fermentation tests were qualitatively similar to those reported in Figure 1.

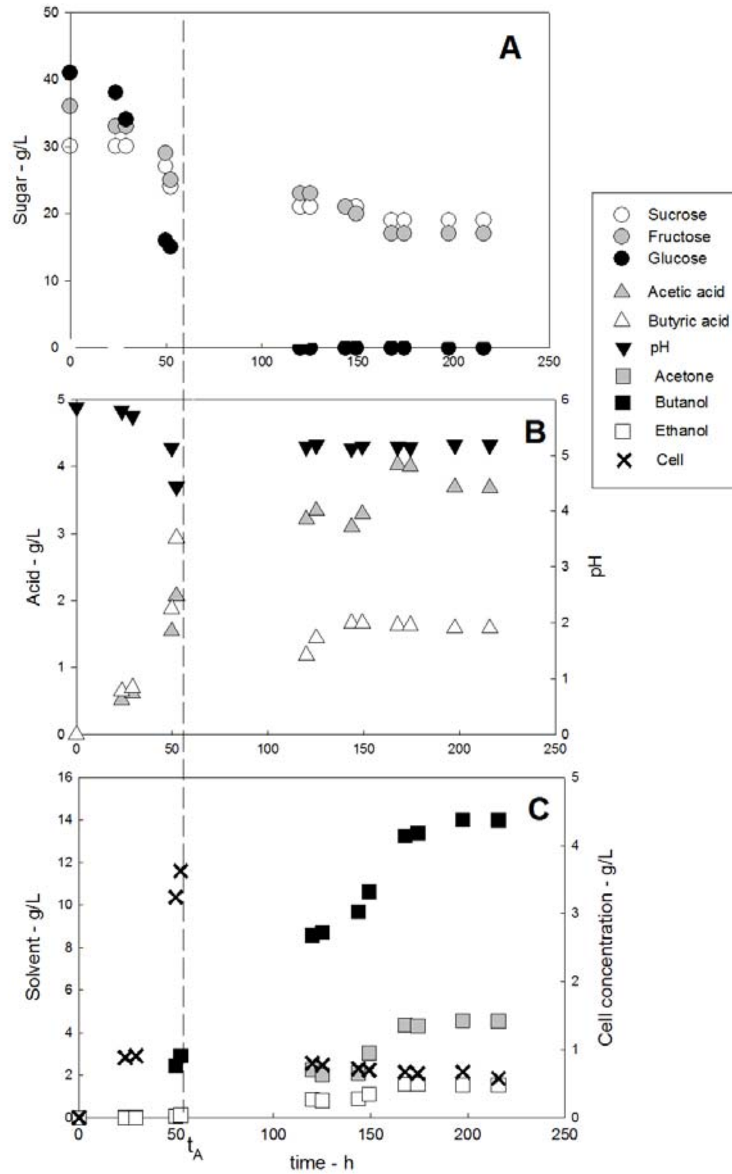

Figure 1: Data measured during C. acetobutylicum fermentation during the batch culture of the apple peels extract (peels to water mass ratio 1/6). The vertical dashed line marks the beginning of the solventogenesis phase.

Figure 2A reports the maximum concentration of butanol, of ABE and of residual acids for the four investigated apple peels to water ratios. Figure 2B reports the conversion of glucose-fructose-sucrose for the four investigated apple peels to water mass ratios.

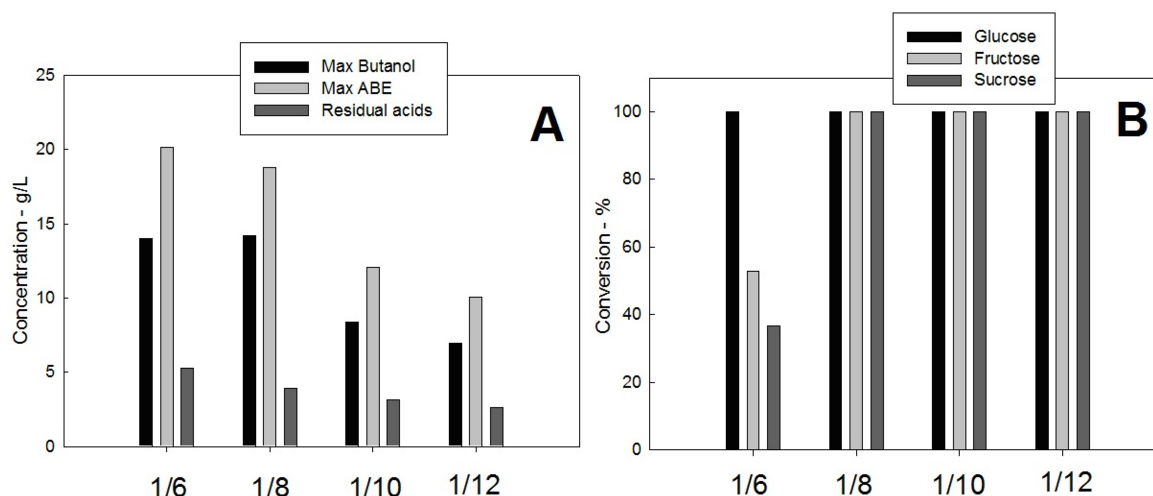

Figure 2: A) Butanol, ABE and residual acid concentration measured at the end of the fermentation test of the apple peels extract for the tested peels to water ratio; B) glucose, fructose and sucrose conversion measured at the end of the fermentation test of the apple peels extract for the tested peels to water ratio.

The analysis of Figures 2 and 3 and of Table 2 points out that:

- glucose was completely converted at the end of the batch tests for all the investigated peels to water mass ratios. Unconverted fructose and sucrose were detected at the end of the batch test carried out with the peels to water mass ratio 1/6. Fructose and sucrose were completely converted at the end of the batch tests carried out with more diluted suspension: peels to water mass ratios lower than 1/6;
- the highest concentration of butanol was about 14 g/L - maximum tolerance value in the case of wild type *C. acetobutylicum* (Jones and Woods, 1986) – and it was measured during the fermentation tests carried out with peels to water ratios 1/6 and 1/8. This concentration was significantly reduced when increasing the dilution of the initial peels suspension;
- the maximum concentration of ABE was about 20 g/L and it was measured during the fermentation tests realized with peels to water ratios 1/6 and 1/8. This concentration was significantly reduced with the dilution of the initial peels suspension;
- the concentration of residual acids was significantly reduced with the decrease of the peels to water ratio.

The reported results highlight that *C. acetobutylicum* was able to metabolize sugars typically present in the extract of fruit peels supplemented with the necessary nutritional factors. In particular, the best results, in terms of butanol produced and conversion of sugars, were obtained for the fruit peels to water mass ratio 1/8.

4. Final remarks

The results of fermentation tests carried out with sugars from fruit peels extracts pointed out that C. *acetobutylicum* is able of utilizing this kind of agro-industrial residues for the production of solvents (ABE). Batch tests were carried out under a wide interval of peels to water mass ratio (1/6, 1/8, 1/10 and 1/12).
The best performances - in terms of produced butanol and conversion of sugars - were obtained for the fruit peels to water mass ratio 1/8. Indeed, peels to water mass ratios lower than 1/6 (1/8, 1/10 and 1/12) were characterized: by total sugar conversion and by low butanol concentration (<8.5 g/L). Tests at peels to water ratios higher than 1/8 (1/6) were characterized by high butanol production (about 14 g/L) and by incomplete sugar conversion.

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References

Ezeji T.C., Qureshi N., Blaschek H.P., 2007, Butanol production from agricultural residues: impact of degradation products on *Clostridium beijerinckii* growth and butanol fermentation, Biotechnol. Bioeng. 97, 1460-1469.

Han S.K., Shin H.S., 2004, Biohydrogen production by anaerobic fermentation of food waste, Int. J. Hydrogen Energy 29(6), 569–77.

Jones D.T., Woods D.R., 1986, Acetone-butanol fermentation revisited. Microbiol. Rev. 50(4), 484-524.

Kandari V., Gupta, S., 2012, Bioconversion of vegetable and fruit peel wastes in viable product. J. Microbiol. Biotechnol. Res. 2(2), 308–312.

Katami T., Yasuhara A., Shibamoto T., 2004, Formation of dioxins from incineration of foods found in domestic garbage, Environ. Sci. Technol. 38(4), 1062–5.

Mamma D., Kourtoglou E., Christakopoulos P., 2008, Fungal multienzyme production on industrial by-products of the citrus-processing industry, Bioresour. Technol. 99(7), 2373-2383.

Mtui Y.S., 2009, Recent advances in pretreatment of lignocellulosic wastes and production of value added products, Afr. J. Biotechnol. 8(8), 1398-1415.

Oberoi, H.S., Vadlani P.V., Madl R.L., Saida L., Abeykon J.P., 2010, Ethanol production from orange peels: Two-stage hydrolysis and fermentation studies using optimized parameters though experimental design, J. Agric. Food Chem. 58(6), 3422-3429.

Parekh M., Formanek J., Blaschek H.P., 1999, Pilot-scale production of butanol by *Clostridium beijerinckii* BA101 using a low-cost fermentation medium based on corn steep water, Appl. Microbiol. Biotechnol. 51(2), 152-7.

Qureshi N., Blaschek H.P., 2000, Economics of butanol fermentation using hyper-butanol producing *Clostridium beijerinckii* BA101, Trans IChemE 78(Part C), 139–144.

Qureshi N., Saha B.C., Dien B., Hector R.E., Cotta M.A., 2010, Production of butanol (a biofuel) from agricultural residues: part I- use of barley straw hydrolysate, Biomass Bioenerg 34(4), 559-65.

Raganati F., Götz P., Olivieri G., Marzocchella A., Salatino P., 2015a, Butanol production from hexoses and pentoses by fermentation of *Clostridium acetobutylicum*, Anaerobe, 34, 146–155.

Raganati F., Procentese A., Olivieri G., Götz P., Salatino P., Marzocchella A., 2015b, Kinetic study of butanol production from various sugars by *Clostridium acetobutylicum* using a dynamic model, Biochemical Engineering Journal 99, 156–166.

Sánchez-Orozco R., Balderas-Hernández P., Flores-Ramírez N., Roa-Morales G., Saucedo-Luna J., Castro-Montoya A.J., 2012, Gamma irradiation induced degradation of orange peels, Energies 5(8), 3051-3063.

Uçkun Kiran E., Trzcinski A. P., Ng W.J., Liu Y., 2014, Bioconversion of food waste to energy: A review, Fuel 134, 389–399.