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Short communication

Co-fermentation of alfalfa juice and hardwood hydrolysate for butanol production in combined biorefinery systems

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ABSTRACT

In this study, a new fermentation medium combining a flocculated hemicellulosic wood hydrolysate (FHWH), rich in carbohydrates, obtained from a pulp and paper mill and an alfalfa juice, rich in nitrogen was used to produce butanol by *Clostridium acetobutylicum* ATCC 842. It was demonstrated that pH control enhances solvent production. The culture containing the two renewable biomass resources resulted in the production of 3.80 g/L of acetone-butanol-ethanol (ABE) with a productivity of 0.03 g/L h and a yield of 0.14 g/g. The obtained results demonstrated that the proposed combination can be used as a new fermentation medium for ABE production. However, the complexity of the medium induced an inhibitory effect and a decrease in the production of ABE, compared to the fermentation of FHWH and yeast extract.

1. Introduction

The use of biomass as a response to the environmental concerns related to fossil resources has emerged as an interesting option and a driving force for the development and implementation of biorefining facilities where biofuels can be efficiently produced (Cherubini, 2010; Cherubini et al., 2009). Among the most promising biofuels, butanol, a product of the Acetone-Butanol-Ethanol (ABE) fermentation, is considered as a renewable alternative to the conventional jet fuel. However, the cost of biomass, which accounts for 35-50% of the total production cost of biofuels, affects significantly the economic viability of the biological butanol production by *Clostridia* species (Green, 2011; Sultana and Kumar, 2011). Forest biomass and agricultural resources such as wheat and barley straw, corn stover, switchgrass (Qureshi et al., 2013) and agro-industrial food waste (Ujor et al., 2014a,b) have been investigated as potential low cost carbon sources. Pulp and paper (P&P) mills can supply sugars rich streams as substrates for butanol production within the concept of the integrated forest biorefinery (IFBR). A previous study has demonstrated that ABE can be produced from hemicellulosic wood hydrolysates extracted from a dissolving pulp mill and detoxified by flocculation or combined nanofiltration and flocculation (Mechmech et al., 2015a).

It should be noted that besides sugars, *Clostridia* species require expensive nutrients such as vitamins and minerals and a source of nitrogen, which are crucial for their cell growth and metabolism. Replacing these components by low cost renewable biomass could reduce the cost of butanol production. In a recent study, the feasibility of using alfalfa juice, an agro-industrial residue from fodder pellets production plants, as a nitrogen source for butanol production has been investigated. It has been demonstrated that alfalfa juice could replace yeast extract and enhance butanol production, if added to a synthetic medium at an optimum C:N ratio (Mechmech et al., 2015b).

As the use of a single biomass source cannot support the total feedstock requirement for butanol production (Sultana and Kumar, 2011), in this study it is proposed to extend the scope of the previous work by combining two biorefinery concepts: the IFBR and the green biorefinery. Thus, the objective of the study is to investigate the feasibility of a simultaneous fermentation for butanol

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Table 1
Kinetic parameters of ABE fermentation and xylose consumption.

Carbon and nitrogen sources	Xylose consumption (g/L)	Productivity (g/L h)	Yield (g/g)	Reference
Xylose + YE[b]	28.2	0.04	0.17	Mechmech et al. (2015b)
Xylose + YE[a]	47.2	0.07	0.15	This work
Xylose + AJ[b]	29.5	0.04	0.17	Mechmech et al. (2015b)
FHWH + YE[b]	24.2	0.04	0.25	Mechmech et al. (2015a)
FHWH + YE[a]	41.1	0.06	0.19	This work
FHWH + AJ[a]	27.7	0.03	0.14	This work
Xylose + YE + 10% v/v AJ[b]	31.8	0.05	0.20	Mechmech et al. (2015b)
FHWH + YE + 10% v/v AJ[a]	42.4	0.06	0.17	This work

AJ: alfalfa juice; FWHW: flocculated hemicellulosic wood hydrolysate containing 0.3 g/L of phenolic compounds; YE: yeast extract.
[a] Controlled pH conditions.
[b] Uncontrolled pH conditions.

production of a flocculated hardwood hemicellulosic hydrolysate from a P&P mill, used as a carbon source, and alfalfa juice, an agro-industrial residue from a pellets production plant, used as a nitrogen source.

2. Material and methods

Green juice was obtained by grinding a second cut of early bloom Canadian alfalfa (*Medicago sativa*) for 5 min and by pressing it using a laboratory hydraulic press at pressure conditions comprised between 4 and 6 MPa for 2 min. Its physiochemical characterization reveals a sugar content of 4.60 g/L, a nitrogen content of 3.16 g/L and 2.00 g/L of phenolic compounds (Mechmech et al., 2015b). Hardwood hemicellulosic pre-hydrolysate obtained from a dissolving pulp mill was subjected to acid hydrolysis with sulfuric acid at 1.5% wt/wt, to convert the oligomeric sugars into monomers, and then detoxified by flocculation using ferric sulfate with a final sugars concentration of 26.7 g/L (Mechmech et al., 2015a).

Clostridium acetobutylicum ATCC 824 was obtained from the American Type Culture Collection (ATCC) and inoculum preparation was carried out as described previously by Mechmech et al. (2015b).

Anaerobic batch fermentation experiments were conducted in 1L bioreactor (New Brunswick's BioFlo®/CelliGen® 115) with a working volume of 0.8 L at a temperature of 37 °C and an initial pH of 6.8. During fermentation, the pH was adjusted to 5 with NaOH solution (2 M). High purity nitrogen was continuously sparged in the reactor at a flow rate of 75 mL/min. In the control experiment, xylose (60 g/L) was used as a carbon source and then replaced by 3.5× diluted flocculated hydrolysate containing 0.30 g/L of phenolic compounds. The final concentration of sugars in the FHWH was raised to 60 g/L by adding synthetic xylose and the total concentration was determined by high performance liquid chromatography (HPLC). Alfalfa juice, 4.4 folds diluted to a nitrogen content of 0.90 g/L, was used as a nitrogen source. The solutions were then sterilized separately at 121 °C for 15 min. Prior to inoculation with 5% (v/v) of active growth inoculum, 8 mL of filtered and sterilized stock solution consisting of buffer, vitamins and minerals were added to the medium (Ezeji et al., 2007). During fermentation, 5 mL samples were periodically withdrawn to analyze sugars, ABE and acids concentration. All fermentation experiments were conducted in duplicates and the results are expressed as an average.

Clostridium growth was monitored spectrophotometrically by measuring the optical density at 600 nm using an UV–vis spectrophotometer (Pharmacia Biotech Novaspec®II). Fermentation products (acetone, butanol, ethanol, acetic and butyric acids) were analyzed by gas chromatography (GC 7890A, Agilent Technologies) and sugars were measured by HPLC (Agilent 1260 system), as described previously (Mechmech et al., 2015a,b). The ABE productivity was computed as the total ABE produced divided by the fermentation time and is expressed in g/L h. The ABE yield was calculated as the total ABE produced divided by the total sugars used and is expressed in g/g.

3. Results and discussion

3.1. Effect of pH control on ABE production using xylose or hemicelluloses hydrolysate as a carbon source

As shown in Table 1, the xylose consumption and productivity in the control experiment were higher than those determined in a previous work, where the same fermentation experiment was conducted under uncontrolled pH conditions (Mechmech et al., 2015b).

Following the control experiment, where xylose and yeast extract were used as a carbon and a nitrogen source, fermentation was carried out at controlled pH conditions with the same nitrogen source, while the xylose was substituted by a hemicellulosic wood hydrolysate. The hydrolysate was previously detoxified by flocculation with ferric sulfate and diluted to reach phenols concentration of 0.30 g/L. The dilution factor and the concentration of the phenolic compounds were selected based on a previous work, aiming to determine the inhibitory concentration and the efficiency of their removal on ABE fermentation (Mechmech et al., 2015a). Thus, the culture produced 7.99 g/L of ABE (Fig. 1), which is 1.32× higher than the total ABE concentration obtained in a previous study, where the same hydrolysate was used as a carbon source under uncontrolled pH conditions. Xylose consumption increased by 41%, which is in agreement with previously reported results by Jiang et al. (2014), demonstrating that a pH control strategy can improve the xylose use due to mechanisms related to the induction of transport systems, glycolytic enzymes and co-factor generation improvement. The concentration of acetic and butyric acids accumulated at the end of the fermentation was 1.66 and 1.60 g/L respectively, compared to 4.48 and 3.08 g/L previously obtained (Mechmech et al., 2015a). In uncontrolled pH conditions, the pH value decreases with the accumulation of undissociated forms of organic acids that can permeate through the cytoplasmic membrane and negatively affect the cells metabolism and the acids re-assimilation for solvent production (Jiang et al., 2014). In addition, the ABE yield was 0.19, compared to 0.17 g/g obtained during the control fermentation, thus confirming that the flocculated hemicellulosic hydrolysate can be used as a renewable substrate for butanol production by *C. acetobutylicum* ATCC 824.

3.2. ABE fermentation using flocculated wood hydrolysate as a carbon source and alfalfa juice as a nitrogen source

A previous study has demonstrated that alfalfa juice can replace yeast extract at a nitrogen content of 0.90 g/L to produce 4.98 g/L of ABE in a synthetic fermentation medium containing xylose as a carbon source (Mechmech et al., 2015b). Thereby, it was