Fuel 143 (2015) 399–403

Contents lists available at ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel

King Grass: A promising material for the production of second-generation butanol

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HIGHLIGHTS

- King Grass was suitable for butanol production using *Clostridium acetobutylicum*.
- Pretreatment with NaOH was successfully used to condition the material for hydrolysis.
- pH control with citrate was counterproductive to butanol yield.
- Butanol yield of 4500 L/ha/year was achieved with King Grass.
- SHF configuration performed better than the SSF configuration.

ARTICLE INFO

Article history:
Received 22 August 2014
Received in revised form 20 November 2014
Accepted 24 November 2014
Available online 4 December 2014

Keywords:
Butanol
ABE fermentation
SHF systems
SSF systems
Lignocellulosic biomass

ABSTRACT

Butanol is an alcohol that can be used as fuel and as intermediate in chemical synthesis. Due to its characteristics, butanol has a very high compatibility with gasoline. Concerns about non-renewable resources and environmental problems associated with petroleum have revived the interest for the biological pathway to produce butanol. Furthermore, the use of lignocellulosic biomass as feedstock has turned very attractive due its high availability. We found that the most suitable grass for butanol production in Colombia, based on availability, plant conditions, theoretical yield to solvents, crop timing and composition was King Grass. Samples of this species were characterized to determine their composition. Then, the material was pretreated by alkaline delignification and hydrolyzed with a commercial cellulase preparation, obtaining a maximum sugar content of 78 g/L. The hydrolysate was fermented with the bacteria *Clostridium acetobutylicum* ATCC 824 in simultaneous and separated hydrolysis and fermentation configurations. Separated hydrolysis and fermentation performed better than the simultaneous configuration. Best results were found when the fermentation was carried out without pH control, i.e.,18 g/L solvents and 10.4 g/L of butanol, which corresponds to 145 L of butanol per ton of pretreated biomass and to a maximum butanol yield of 4500 L per hectare per year.

1. Introduction

Non-renewable character of most of the energetic resources has renewed the interest in technologies that use renewable resources and eventually could replace those obtained from petroleum, coal and natural gas. In this context, lignocellulosic biomass is presented as a promissory alternative due to its abundance, availability and relative low cost [1]. Also lignocellulosics are not edible crops and do not compete directly with the food supplies when marginal lands are used for its harvesting or when residues are used [2].

Butanol is an alcohol that could be used as a fuel, because it has interesting characteristics like low water miscibility, low vapor pressure, energy content and octane number close to gasoline [3]. Butanol is also a raw material for the chemical industry and is used in the manufacture of butyl phthalate, butyl acrylate, glycerol ethers and as an industrial solvent [4].

Nowadays most of the butanol is produced from crotonaldehyde dehydrogenation and from hydroformilation of olefins (OXO process) which come from oil. However, it is possible to

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http://dx.doi.org/10.1016/j.fuel.2014.11.077
0016-2361/

obtain butanol via fermentation using certain species of bacteria, in this fermentation, butanol is the main liquid product of the process, but acetone and ethanol are also obtained, the mixture of these three products are commonly known as solvents. In the first half of XX century butanol was produced from the fermentation of corn, molasses, and starch [4] but those processes were forsaken in favor of petrochemical ones because of their better profitability [5].

Lignocellulosic materials are an alternative with high potential for butanol production, but they require a previous process to obtain monomeric sugars from cellulose and hemicelluloses that compose them. This process is not required when sucrose or starch are used as raw materials.

Before the hydrolysis step, pretreatment is necessary. The main target of the pretreatment is to cleave the lignin/cellulose unions, solubilize lignin and alter the crystalline structure of the material [6]. This is necessary to condition the biomass to the hydrolysis step because enzymes are not capable to access efficiently the lignin cover and reach the cellulosic fraction. Hydrolysis is a process carried out with enzymes called cellulases, which consist in three kinds of enzymes: endoglucanases, exoglucanases and β-glucosidases. The synergic action of these enzymes converts the cellulose in glucose. The reaction is carried out under mild conditions (40–50 °C, 4–5 pH, 1 atm) and it has some advantages like the no production of inhibitors, low equipment corrosion and high sugar yield. It also has some disadvantages such as high costs of enzymes and long reaction times compared to acids [7].

According to the mentioned above, even when lignocellulosics have lower prices than sugar and starch based crops, the cost of transforming them to raw materials suitable to fermentation process has been historically too high to attract industrial interest and it is necessary to develop processes with high productivity to make the process viable.

Qureshi [8,9] reported the use of agroindustrial wastes and a grass (Switchgrass) for the production of butanol. Acid pretreated and enzymatic hydrolyzed Switchgrass gave poor results (less than 2 g/L of solvents) and only by supplementing the hydrolysate with glucose (35 g/L), 14.6 g/L solvents and 9.55 g/L of butanol were obtained, but those results are just a consequence of the supplemented glucose.

The use of King Grass for butanol production has not been reported yet but its characteristics like harvesting times and productivity are very attractive. King Grass (*Pennisetum hybridum*) is a gramineae adapted to tropical conditions with a wide distribution of rain conditions and soil fertility, including low fertility acidic soils. The species is perenne and can grow up to 3 m height. The plant has achieved good adaptability and grows in high fields and with prolonged dry conditions. The yield of the crop is about 60–80 ton of dry biomass per year [10,11].

In this work, the production of butanol, by fermentation with *Clostridium acetobutylicum* ATCC 824, from the lignocellulosic King Grass pretreated with sodium hydroxide and hydrolyzed with a commercial cellulases pull is reported. High yields of butanol were obtained without supplementing sugars to the media.

2. Materials and methods

2.1. Materials

The lignocellulosic King Grass was obtained from a local farm located in Medellín Colombia (1500 meters over sea level), and harvested between October and January. This grass was dried in air for easy handling and transport, then was grinded to particle size less than 3 mm and dried again to achieve a moisture content of less than 10% (wt.). The material was characterized to determine cellulose, hemicellulose, lignin, extractives and ash contents in the solid.

Industrial sodium hydroxide (purity 96%) was used in the pretreatment, sulfuric acid (Merck 97%) was used for neutralization. RCM broth and reagents for media preparation were purchased from Merck, citric acid and citrate for buffer solution preparation were from Panreac.

2.2. Pretreatment and hydrolysis

Alkaline hydrolysis was chosen as the pretreatment to condition the raw material for the hydrolysis step. Conditions were extracted from an optimization carried out in our laboratory [12]. Those conditions were 120 °C, 1 h, 2% of NaOH solution and liquid /solid ratio of 20(w/w). 50 g of the material was poured into 2 L erlenmeyer flask, the solution of sodium hydroxide was added and the erlenmeyer was sealed and put in the reactor and left during corresponding time at the fixed conditions. After that, the liquid was separated from the solid using a filtration device. The solid was neutralized, dried and stored in plastic bags.

For the hydrolysis step the commercial cellulase preparation Acellerase 1500 (Genencor®) was used. Hydrolysis was carried out adding the commercial enzyme accellerase 1500 with a solid loading of 10% and a enzymatic loading of 30 FPU. Citrate buffer pH 4.8 (0.05 M) is recommended to perform hydrolysis, but it could not be used in the fermentation experiments due to the interferences with the pH control of the microorganism (see Section 3). Rather, deionized water was used.

2.3. Fermentation

The microorganism used was *Clostridium acetobutylicum* ATCC 824 (ATCC® Rockville, USA). The liophilizated was activated following the manufacturer instructions and then it was cryopreserved in silica and glycerol. The inoculum was prepared adding the cryopreserved in a glass vial with RCM (Reinforced clostridia media, MERCK), incubated at 37 °C until optical density of 0.8–1.2 were achieved, and then used for fermentation. All the fermentation were supplemented with the medium reported by Monot [13]. High purity nitrogen gas was sparged at the beginning of fermentations to guarantee the anaerobiosis in the process. A blank run using glucose (60 g/L) as carbon source was carried out.

To evaluate the effect of pH, some experiments were carried out with and without the addition of buffer solution to the media. The buffer solution consisted in a mix of sodium citrate and citric acid 0.05 M with pH of 4.8.

For the SHF (separate hydrolysis and fermentation) the material was left in hydrolysis for 24 h at 50 °C and 180 rpm, then enzyme was denaturalized heating the system to 90 °C. Then the hydrolyzated was supplemented with Monot media and inoculated. Fermentation was carried out at 37 °C and 50 rpm in a shaker incubator (Innova 40 New Brunswick Scientific).

For the SSF (simultaneous hydrolysis and fermentation) the material was left in hydrolysis 12 h at 50 °C and 180 rpm. Then, it was supplemented with Monot media and inoculated. Fermentation was carried out at 37 °C and 50 rpm in a shaker incubator.

The attempts for fermenting the liquid fraction from the pretreatment (black liquor) were unsuccessful and are discussed in the results section.

2.4. Analyses

Materials were characterized following the protocols of the National Renewable Energy Laboratory (NREL) [14–16] for determination of ash, moisture and extractives in biomass. Cellulose, hemicellulose and lignin contents in pretreated and non-pretreated materials were determined by UV–vis spectroscopy after acid hydrolysis with 72% H_2SO_4. Total reducing sugars