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The potential of agricultural banana waste for bioethanol production

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ABSTRACT

Banana is one of the most important fruit crops around the world. After harvesting, it generates large amounts of lignocellulosic residues that could be used for second generation ethanol production. Optimal operating conditions of saccharification and fermentation processes, of the two main agricultural wastes of banana crop: pseudostem and rachis, for bioethanol production were selected in order attain conditions transferable to industry (optimizing resources and time). Both materials were previously pretreated with acid-catalyzed steam explosion. Full factorial experimental design was used in order to determine enzymatic hydrolysis process conditions to obtain glucose concentrations between 75 and 100 $g.L^{-1}$. Optimal enzymatic hydrolysis conditions for rachis and pseudostem were 17.6% of solid loading and 16.0 $FPU.g^{-1}$ glucan of enzyme dosage and 15.1% of solid loading and 14.9 $FPU.g^{-1}$ glucan of enzyme dosage, respectively. The most suitable configuration to attain the highest volumetric ethanol productivity for rachis was 8 h prehydrolysis followed by a Simultaneous Saccharification and Fermentation (PSSF 8 h). Meanwhile, for pseudostem, higher ethanol production was obtained in Simultaneous Saccharification and Fermentation (SSF) configuration. At optimized conditions, pseudostem reached 112 $L.t^{-1}$ of ethanol, and rachis 103 $L.t^{-1}$. These conditions led to achieve ethanol production process at high solid loading, low enzyme dosage, low yeast inoculum, no mineral salts supplementation and maximum ethanol productivities.

1. Introduction

Ethanol has been recognized as a suitable alternative to partially replace fossil fuels in transportation sector [1]. In 2015, ethanol production increased by 4% globally, with record production levels despite the fact that oil hit historic low prices at the end of that year [2]. Indeed, 2015 was an outstanding year for bioethanol because second generation (2G) lignocellulosic ethanol took off at commercial stage [3]. Second generation ethanol is one of the most promising alternatives to fossil fuels because it can reduce fossil fuel dependence [4]. Consequently, greenhouse gas emissions will reduce [5], mitigating climate change effects.

Lignocellulosic biomass is a promising feedstock for ethanol production because of its abundance and high availability. Indeed, agricultural residues can be used locally and do not trigger a fuel vs. food competition. Vast information is available about the optimization of bioethanol production process from lignocellulosic waste of staple crops like wheat straw, corn stover, rice husk, etc. However, little attention has received lignocellulosic biomass from non-staple crops, although big areas are dedicated to its farming around the world. Banana is the most important fruit crop in the world, in terms of metric tons harvested [6]. In 2014, the production reached 114.1 million t, mostly concentrated in two continents: 56% occurred in Asia and 26% in the Americas [6]. Banana plant produces a bunch of fruits once in its life cycle, generating large amount of lignocellulosic residues. In fact, for each t of banana produced, 2.13 t of fresh lignocellulosic residue (pseudostem, leaves and rachis) is generated [7]. Normally, pseudostem and leaves are left over the field and rachis is gathered in the packing plant causing environmental problems such as the spread of diseases or polluting groundwater [8] because these residues have little or no productive use.

Some studies have been focused on the analysis of agricultural waste of banana, and most of them have studied fruit waste and peel bioconversion into ethanol [9,10]. Meanwhile, banana lignocellulosic biomasses have received little attention. For example, Baig et al. reported the effect of time, pH, temperature and particle size on the saccharification and fermentation process of a mixture of the main agricultural banana wastes [11]. Other authors studied the production of bioethanol using cellulolytic, thermophilic and ethanologenic bacteria from banana leaves [12]. However, to the best of our knowledge, no information is still available in scientific literature about the optimal conditions for enzymatic conversion of lignocellulosic banana wastes

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into fermentable sugars, and the most suitable fermentation configuration for further transformation into ethanol.

The production of bioethanol from lignocellulosic biomass involves four main steps: feedstock pretreatment, enzymatic saccharification, fermentation and product recovery. The final step is crucial to make the process economically viable at a commercial scale because of the high steam energy consumption due to low ethanol titer when lignocellulose materials are used as feedstock. To achieve economic and energy benefits in the distillation step, ethanol titer of fermentation broth should be $\geq 40\ g.L^{-1}$, which implies that glucose yields, after enzymatic hydrolysis, must be at least $80\ g.L^{-1}$ (w/w) [13], considering theoretical ethanol yield (0.511). To reach this higher ethanol concentration, high solids loadings during hydrolysis of lignocellulose are needed. However, due to the reduced enzymatic hydrolysis effectiveness at high biomass solid loading, higher amounts of enzymes are typically required to obtain reasonable sugar concentration and, consequently, high ethanol titers. This fact increases operating costs of the bioconversion process of lignocellulose into ethanol. In this context, it is necessary to establish the optimal operating conditions to reach at least $40\ g.L^{-1}$ of ethanol at reasonable enzymatic dosage to reduce the costs of distillation.

Several processing configurations can effectively transform lignocellulosic carbohydrates into sugars and ferment them into ethanol, reducing operating costs and time. Here are listed some of them. Separate Hydrolysis and Fermentation (SHF), in which enzymes hydrolyze the solids, and sequentially, yeasts ferment the released sugars. The major advantage of this configuration is that, operating in different vessels, enzymes and microorganisms can both work at optimal conditions of temperature and pH [14]. An alternative approach is the Simultaneous Saccharification and Fermentation (SSF) process, where fermenting microorganisms consume the glucose produced by the hydrolyzing enzymes as they are released. The benefits of this integrated process are the reduction of enzyme inhibition by final products through their immediate conversion into ethanol, and the fact that SSF acts in one reactor so the investment cost for processing plant is decreased [15]. A variation of SSF configuration is the Pre-hydrolysis and Simultaneous Saccharification and Fermentation (PSSF) process, where the substrate is exposed to a previous hydrolysis for a short period before yeast inoculation.

This work aims to define the most appropriate conditions for enzymatic hydrolysis and fermentation of steam exploded banana agricultural wastes (rachis and pseudostem) to reach, in saccharification stage, glucose concentration $\geq 100\ g.L^{-1}$ and final ethanol concentration $\geq 40\ g.L^{-1}$ in fermentation stage. Since increasing solid loading in hydrolysis and fermentation step is one of the most important challenges to produce ethanol in a more economic way, effectiveness of different configuration processes were compared to successfully achieve the minimum operating requirements to produce lignocellulosic ethanol at industrial conditions.

2. Materials and methods

2.1. Pretreated material

The Canarian Institute of Agricultural Research (Tenerife, Spain) kindly provided banana lignocellulosic wastes (rachis and pseudostem) from the cultivar *Musa acuminata* Colla (AAA) 'Dwarf Cavendish'. Rachis (26.1% cellulose, 11.2% hemicellulose, 10.8% lignin, 29.9% ash and 18.2% extractives) and pseudostem (20.1% starch, 20.1% cellulose, 9.6% hemicellulose, 10.1% lignin, 18.5% ash and 14.7% extractives) were milled separately, to pass a 10 mm mesh size (moisture content ≈10%). Then, it was subjected to an acid-catalyzed steam explosion pretreatment in a 2 L steam explosion pilot plant described in Ballesteros et al. [16]. The applied conditions were chosen based on a previous work developed for optimization of sugar production [17]. For pseudostem, the conditions were 177 °C, 5 min of residence time and 2.2% H_2SO_4 (v/v), presenting a high overall glucose yield of 91.0%. Meanwhile, the conditions for rachis were 198 °C, 5 min and 1.5% H_2SO_4 (v/v) with an overall glucose yield of 87.1%. After the steam explosion, the slurry was recovered and vacuum filtered to separate the liquid fraction (prehydrolyzate) and the Water Insoluble Solid (WIS) fraction. Afterwards, the liquid fraction was analyzed for sugars and total phenols; and the WIS (unwashed) was analyzed for sugar, lignin and ash content, as described in Section 2.4. Subsequently, it was stored at 4 °C until use.

Table 1
Experimental design for the optimization of the enzymatic hydrolysis.

N	Solid load (%)	Enzyme dosage (FPU)
1	10	7.5
2	10	15
3	10	22.5
4	15	7.5
5	15	15
6	15	22.5
7	20	7.5
8	20	15
9	20	22.5

2.2. Enzymatic hydrolysis

Enzymatic Hydrolysis (EH) analysis was performed with the unwashed and wet WIS of each biomass (calculations were performed in dry basis) because water wash decreases the sugar content due to the removal of soluble sugars that may have remained after the separation of both fractions. An experimental design was developed following a 3^2 factorial design. The variables considered were enzyme dosage ($7.5–22.5\ FPU.g^{-1}$ glucan) and solid loading (10–20% w/w) (See Table 1). The selected ranges included values near to the ones expected to be used in the industry [18]. This infers low enzyme dosage ($< 20\ FPU.g^{-1}$ glucan) and high solid loading (≥15%) [13]. Novozymes kindly provided the enzymes used in this work, which were Celluclast 1.5 L with an activity of 69 filter paper units ($FPU.mL^{-1}$) and Novozym 188 with an activity of 530 international units ($IU.mL^{-1}$). Enzyme activities were measured following standard procedures according to the National Renewable Energy Laboratory analytical procedure NREL/TP-510-42628.

Experiments were carried out in 250 mL glass bottles with a total reaction volume of 50 mL. In each bottle the desired amounts of WIS, enzymes, water and Na-citrate buffer (0.05 M pH = 4.8) were added. The bottles were placed in a STR4 rotator drive (Bibby Scientific Limited, Stone, UK) at 18 rpm, and incubated in stove at 50 °C for the first hours of reaction. This novel horizontal agitation system provides a rotating drum mixer effect, maximizing the contact surface between biomass and enzymes in the first hours of reaction. Considering that at high solids loadings the reaction mix has a pasty texture, this type of agitation improves the rheology of the medium, allowing improving hydrolysis yields. After this time, the flasks were transferred to an orbital shaker.

Samples were taken at 0, 4, 8, 24, 48, 72 and 96 h; centrifuged (13,000 rpm, 5 min) and the supernatant was used for sugar content analysis following the analytical method described in Section 2.4. All experiments were carried out in triplicate.

2.3. Fermentation configuration testing

2.3.1. Microorganism and growth conditions

Saccharomyces cerevisiae Ethanol Red (Fermentis, France) was used for fermentation assays. For inoculum preparation, cells were grown in 100 mL Erlenmeyer flasks containing 0.1 g yeast extract, 0.75 g glucose, 0.05 g ammonium sulfate, 0.025 g monobasic potassium phosphate,