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*Published in:*
Biomass Conversion and Biorefinery

*DOI (link to publication from Publisher):*
10.1007/s13399-018-00367-0



*Publication date:*
2019

*Document Version*
Publisher's PDF, also known as Version of record

Biomass Conversion and Biorefinery (2019) 9:553–563
https://doi.org/10.1007/s13399-018-00367-0

ORIGINAL ARTICLE

# Bioethanol potential of raw and hydrothermally pretreated banana bulbs biomass in simultaneous saccharification and fermentation process with *Saccharomyces cerevisiae*

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Received: 11 October 2018 / Revised: 14 December 2018 / Accepted: 18 December 2018 / Published online: 19 January 2019


**Abstract**
Residual banana bulbs (RBB) were characterized and assessed as a potential starch and cellulose-based feedstock for bioethanol production. To facilitate the enzymatic digestibility, hydrothermal pretreatment was performed on RBB prior to simultaneous saccharification and fermentation (SSF) with *Saccharomyces cerevisiae*. Composition of RBB was similar to traditional starch and cellulose-based feedstocks with high glucan (60 g/100 gDM) and relatively low lignin content (7 g/100 gDM). Both amylase and cellulase were needed to efficiently hydrolyze RBB. The highest ethanol yield (310 kg EtOH/ton_DM_RBB, 93% of theoretical production based on total available glucose) was obtained with non-pretreated RBB. SSF can be carried out at lower RBB concentrations. Hydrothermal pretreatment affected negatively the bioethanol potential due to the loss of fermentable carbohydrates. In a case study of an African leading producer of bananas and plantains (Cameroon), the energy derived from bioethanol was 80 GWh ethanol/year and corresponded to 1.6% of the annual transportation requirement. This study shows that RBB is a promising alternative feedstock for bioethanol production.

**Keywords** Banana bulbs · Hydrothermal pretreatment · Bioethanol · Fermentation · Biorefinery

## 1 Introduction

Banana (*Musa acuminata* and *Musa balbisiana*) is an important crop widely cultivated in Asia (continent of origin), South America, Caribbean countries, and Africa, where its fruit contributes to food security and socio-economical stability. Currently, banana is the second most produced fruit in the world with a worldwide annual production of approximately

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125 million tons [1, 2], and which generates about 250 million tons of fresh lignocellulosic biomass residues [3]. In Cameroon, the production of bananas and plantains represents the second agricultural economic resource of the country after wood [1]. For the year 2012, banana production reached 1.4 million tons in Cameroon, resulting in about 90,000 tons as dry matter of post-harvest agro-industrial residues such as pseudo-stems, peduncles, bulbs, leaf sheath, and rachis [4]. Among these post-harvest residues, banana bulbs represent a potential renewable feedstock for a variety of biorefinery applications, thanks to its specific composition approximately 50% of starch and 20% of lignocellulose, on a dry matter basis (Awedem et al. (unpublished results)).

Bioethanol is nowadays increasingly used as an alternative liquid biofuel for transportation in high- and medium-income countries like the USA and Brazil, respectively [5, 6]. Both countries produce a total of about 16 billion liters of ethanol per year mainly from sugar cane (*Saccharum L.*) and corn (*Zea mays L.*) as feedstocks [6]. However, the use of these important sources of food and feed for biofuel production is currently criticized due to the use of arable lands for fuel production to the detriment of food production. The current increased worldwide ethanol demand in transportation sector will lead to the expansion of fuel ethanol production using these crops and could consequently lead to shortages and price increase in food and feed [7]. Using post-harvest wastes like residual banana bulbs as a feedstock for ethanol production could be an effective alternative. Banana bulbs are non-food biomass resources and therefore do not compete with human food supply. When compared to other post-harvest banana biomass wastes (e.g., banana rachis, pseudo-stems, peduncles, leaves, etc.) that are visually green and fibrous, banana bulbs are rather white and less fibrous. Banana bulbs are one of the main residues (11% of the total dry residues) of the large quantities of post-harvest banana wastes [8]. They have a good potential as a raw material for ethanol production because of their apparent high starch content and low lignin content that can be hydrolyzed and fermented to produce ethanol. Despite the availability and the attractive chemical composition of banana bulbs biomass for a variety of biorefinery applications, their bioethanol potential has not been published in the scientific literature.

On the one hand, ethanol production from starchy feedstocks by conventional fermentation requires saccharification with hydrolytic enzymes after low temperature (< 90 °C) pretreatments [9] and subsequent fermentation using the yeast *Saccharomyces cerevisiae* (*S. cerevisiae*). On the other hand, ethanol production from lignocellulosic feedstocks requires physico-chemical pretreatment prior to fermentation to decrease the recalcitrance of the biomass by breaking up the lignocellulosic structure of the pretreated materials and thus enabling enzymatic hydrolysis. These pretreatments usually require high severity and temperature to initiate the deconstruction of biomass [5, 10–13]. Ethanol production from a starch and cellulose-based feedstock like banana bulbs biomass would require a compromise between low- and high-temperature pretreatment. When compared to other pretreatment processes like chemical (acid and alkaline) pretreatments that are expensive, hydrothermal pretreatment has been proven to be an effective and cost-efficient method for a wide range of starch and cellulose-based materials including cassava pulp and corn, producing highly digestible fiber fractions [5–7, 9, 11, 14, 15]. Hydrothermal pretreatment fulfills the following potential advantages as compared to chemical (acid and alkaline) pretreatments: (1) no chemicals (or only small amounts of mineral acids/alkali as catalysts) are required, (2) hemicelluloses are converted into soluble compounds (usually, a mixture of mono- and oligosaccharides), and (3) the pretreated solid (enriched in cellulose and lignin) usually present high susceptibility for subsequent enzymatic hydrolysis [5, 10, 14–16]. Moreover, hydrothermal pretreatment as compared to chemical (acid and alkaline) pretreatments shows favorable features in terms of environmental impact, selectivity of component separation (hemicelluloses can be dissolved without causing significant effects on cellulose and acid-insoluble lignin), limitation of the residence times, limitation of chemicals and sludges, limitation of equipment corrosion, and reduced capital and operational costs [16–21]. Hydrothermal treatment is defined commonly as “reactions occurring under the conditions of high temperature and high pressure in aqueous solutions in a closed system” [14]. The two forms of hydrothermal pretreatment are liquid hot-water pretreatment and steam explosion. However, steam explosion is known to lead to the loss of some part of biomass during the explosive decomposition [8, 22]. Liquid hot-water (LHW) treatment is a traditional hydrothermal pretreatment practice. As other pretreatment processes, LHW is known to generate fermentation inhibitors during the process. However, *S. cerevisiae*, the most commonly used microorganism for ethanol production [9, 23], has been found to resist such fermentation inhibitors [5, 6, 24–28]. After hydrothermal pretreatment, the fiber fractions left are mainly composed of cellulose, hemicellulose, and starch that can be enzymatically hydrolyzed to release monomeric sugars, which subsequently become available for microbial conversion into ethanol. Different hydrolytic enzyme mixtures are generally used based on the main components of the feedstocks.

The present research investigates banana bulbs biomass as a potential starch and cellulose-based bioethanol feedstock. A hydrothermal process was chosen as a pretreatment method applied prior to enzymatic hydrolysis in order to increase the efficiency of both hydrolysis and alcoholic fermentation. The effect of hydrothermal pretreatment and the use of enzyme mixture (amylase and cellulase) on bioethanol potential were investigated. We assessed the influence of both enzyme loading and substrate concentration on ethanol yield. The obtained

results were used to determine the renewable energy potential as bioethanol from residual banana bulbs biomass in Cameroon.

## 2 Materials and methods

### 2.1 Raw material

Residual banana bulb biomass (RBB) was collected from an industrial banana plantation (*Plantations Haut Penja*, PHP) in Cameroon after the mature fruits had been harvested. The variety selected for this study was "Grande Naine" (*Musa AAA group*) as extensively described previously [29–31]. The harvested biomass was chopped using a garden mill into particles with maximum diameter of 25 mm. The samples were then oven-dried at 50 °C until constant weight, resulting in a 105 °C dry matter (DM) content of approximately 95% *w*/*w*. Dried samples were ground to particle size of less than 2 mm using FOSS CYCLOTEC_1093 SAMPLE MILL. Ground samples were stored in air-tight plastic bags until use.

### 2.2 Simultaneous saccharification and fermentation

#### 2.2.1 Enzymes used for SSF

Cellulase (commercial Cellic CTec2; activity: 74 FPU/ml_cellulase) and amylase (endo-alpha-amylase–Termamyl; activity: 14 μmol_alpha_1_4_glucose_bond_hydrolyzed/min/ml_amylase) were kindly supplied by Novozymes A/S, Denmark.

#### 2.2.2 SSF reference protocol

About 5 gDM of substrate was mixed with 50 ml of distilled water (pH adjusted at 4.8 with 0.01 M $H_2SO_4$) in 250-ml Schott Duran GL 45 bottle flasks. The mixture was then supplied with 1 ml of cellulase + 1 ml of amylase and incubated for 24 h at 50 °C under constant orbital shaking (300 rpm) as a pre-hydrolysis step. This enzyme mixture was based on the manufacturers (Novozymes A/S, Denmark) recommended loading range and will be further referenced as loading "C".

After the pre-hydrolysis step, 0.2 g of active dry commercial yeast (*Saccharomyces cerevisiae* 95% DM, Malteserkors tørgær, De Danske Spritfabrikker A/S, Denmark) [9, 17, 23, 32, 33] was added along with 0.2 ml of a 24% urea solution as nitrogen source. The flask headspace was flushed with nitrogen gas for 1 min and was secured with glycerol-filled yeast locks, to ensure anaerobic conditions, while enabling carbon dioxide release. The fermentation process was performed at 32 °C, with constant agitation (300 rpm) for 5–7 days. The pH was not further controlled. The flasks were weighed daily to measure the weight loss (caused by $CO_2$ release) and thus monitor fermentation. All SSF experiments were performed in duplicate, with a parallel reference experiment without any addition of enzymes.

At the end of fermentation, the total concentration of ethanol produced was determined simultaneously with carbohydrates by high-performance liquid chromatography (PerkinElmer series 200a). The Aminex HPX-87H column (Bio-Rad) at 65 °C using 0.005 M $H_2SO_4$ as the mobile phase (eluent) with a flow rate of 0.6 ml/min and refractive index detector (RID) were used to determine the concentrations of ethanol produced and carbohydrates (glucose, xylose, and arabinose).

#### 2.2.3 Influence of enzyme activity

SSF of RBB was performed using both the enzyme mixture of the reference protocol (loading "C") and individual enzymes, i.e., (1) RBB + 1 ml of cellulase only and (2) RBB + 1 ml of amylase only.

#### 2.2.4 Influence of enzyme loading

The influence of enzyme loading on SSF was assessed with 5 gDM_RBB as described in the reference protocol, with reduced enzyme loading: (1) 0.5 ml of cellulase + 0.5 ml of amylase, further referenced as "A", and (2) 0.75 ml of cellulase + 0.75 ml of amylase, further referenced as "B".

#### 2.2.5 Influence of substrate concentration

The reference protocol was performed with 5, 2.5, and 1.25 gDM RBB, further referenced as "RBB", "½ RBB", and "¼ RBB", respectively, and with amylase only, keeping the total amylase activity and other volumes unchanged. The removed substrate was replaced by distilled water (pH adjusted at 4.8 with 0.01 M $H_2SO_4$).

#### 2.2.6 Influence of hydrothermal pretreatment on fermentation

SSF of solid fractions recovered after hydrothermal pretreatment was performed following the reference protocol as described above. The fermentation of solid fractions recovered after hydrothermal pretreatment was also performed using their corresponding hydrothermally liquid fractions (pH adjusted at 4.8 with 0.01 M $H_2SO_4$) instead of distilled water as fermentation medium.

### 2.3 Hydrothermal pretreatment

Hydrothermal pretreatment experiments were performed in a Roth high-pressure laboratory autoclave (Model II 300 ml, Carl Roth GmbH Company, Karlsruhe, Germany) fitted with

heating mantle and a magnetic stirrer head MRK 10. About 5 gDM of biomass (RBB) and 95 g of distilled water were loaded to the reactor. Pretreatment was performed at 100, 110, or 120 °C for 10 min once the desired temperature was reached (within 5 to 8 min). These pretreatment conditions were selected as upper range of investigation in order to preserve starch from thermal degradation. It was based on preliminary analysis which had shown 50 g starch/100 gDM RBB (Awedem et al., unpublished results). After the pretreatment, the reactor was cooled in ice cold water for 10 to 15 min. The pretreated materials were separated by filtration on 1-mm mesh size sieve to solid fraction (fibers) and liquid fraction (filtrate). The DM content of the recovered solid fractions was 12.4–12.9 gDM/100 g FM (FM: fresh matter). Both fractions were kept in a freezer at − 20 °C and used for further investigations (sugar analysis, pretreatment by-products, simultaneous saccharification, and fermentation).

## 2.4 Chemical characterization

### 2.4.1 Dry matter and ash content

The dry matter (DM) content of the samples (solid and liquid material) was determined after drying at 105 °C until constant weight. The dry residue was subsequently burned in a furnace at 550 °C for 5 h to determine the total ash content.

### 2.4.2 Determination of extractives

Water and ethanol extractives were removed prior to the determination of the chemical composition analysis of residual banana bulbs (RBB). About 5 g of dry biomass was loaded into a cellulose thimble and the extraction was carried out with 200 g of the distilled water for 12 h in a Soxhlet apparatus [34, 35]. The thimble was then dried and weighed to determine the water-insoluble residue. The extraction was repeated with 200 g of ethanol. Number of siphon cycles per hour was set to 3 for water extraction and 6 for ethanol extraction. Water and ethanol-soluble extractive (total and non-volatile) content in the biomass was calculated using Eqs. (1) and (2).

$$\text{Nonvolatile extractives (NE)}\left(\frac{\text{g}}{100\ \text{gDM}}\right) = \frac{W_{\text{dried water or ethanol extract}}}{\text{W}_{\text{RBB}}} \times 100 \quad (1)$$

where $W_{\text{dried water or ethanol extract}}$ = weight of the extract (evaporated to dryness) (g); $W_{\text{RBB}}$ = dry mass of the initial RBB (g)

$$\text{Total extractives (TE)}\left(\frac{\text{g}}{100\ \text{gDM}}\right) = \frac{W_{\text{RBB}} - W_{\text{dried extracted biomass}}}{\text{W}_{\text{RBB}}} \times 100 \quad (2)$$

where $W_{\text{dried extracted biomass}}$ = weight of the extractives-free biomass remaining in thimble and dried (g).

### 2.4.3 Determination of carbohydrates and acid-insoluble lignin (Klason lignin) in solid material

To quantify carbohydrates and lignin in the extractives-free material (from RBB or solid fractions recovered after hydrothermal pretreatment), a two-step strong acid hydrolysis procedure based on the National Renewable Energy Laboratory (NREL) protocol was performed [34]. About 0.16 g of dried sample was treated with 1.5 ml of 72% (*w*/*w*) sulfuric acid at 30 °C for 1 h, and then, the solution was diluted with deionized water to achieve a 4% (*w*/*w*) sulfuric acid concentration. Diluted samples were autoclaved at 121 °C for 1 h. The hydrolysates were filtered through fritted ceramic crucibles, and the Klason lignin content was determined as the weight of the acid-insoluble residue after drying the fritted ceramic crucibles with retained solids at 105 °C for 12 h. The hydrolyzates were analyzed for sugars (glucose, xylose, and arabinose) using high-performance liquid chromatography (PerkinElmer series 200a) as described above for ethanol analysis. The concentrations of the sugars were expressed as their polysaccharide form (glucose in the form of glucan, etc.). Conversion of sugars to polysaccharides was calculated with their dehydration factor of 0.88 for pentoses and 0.90 for hexoses. The concentrations were also corrected for any degradation that may have occurred during the acid hydrolysis steps using a recovery factor calculated from replicates spiked with known concentrations of the sugars analyzed (g/l).

### 2.4.4 Determination of carbohydrates and degradation products in hydrothermal liquid fraction

Monosaccharides in hydrothermal liquid fraction were directly analyzed by HPLC as described above. To check for the presence of polysaccharides, an 8% (*w*/*w*) $H_2SO_4$ solution was added to the liquid fraction to reach a final concentration of 4% (*w*/*w*) $H_2SO_4$. The acidified liquid fractions were then hydrolyzed at 121 °C for 10 min. The total glucose, xylose, and arabinose concentrations were quantified by HPLC as described above.

Furfural and hydroxy-methyl-furfural (HMF) were measured in the liquid fractions recovered after hydrothermal pretreatment using an Agilent HPLC (Agilent 1260 Infinity Bio-inert Binary LC) equipped with a Hypersol Gold column (Thermo Scientific). The column temperature was 30 °C.

The solvent A was 90% water with 1% acetic acid and 9% methanol and solvent B was $CH_3CN$ (HPLC grade solvents). The gradient was as follows: 0–5′ 0% B, 5–10′ linear gradient up to 100% B, 10–15′ 100% B, 15–20′ linear gradient down to 0% B, 20–30′ 0% B. A UV detector was used to determine the concentrations of furfural and HMF at wavelength of 280 nm. Solutions of known concentration were prepared from 2-furaldehyde (2F, Sigma–Aldrich, ref.181100250) and 5-(hydroxymethyl)-2-furaldehyde (5-HMF, Sigma–Aldrich, ref.121460010) and used for calibration.

#### 2.4.5 Ethanol yield calculation

The theoretical (stoichiometric) conversion from glucose to ethanol is 0.51 g ethanol/g glucose. Ethanol yield (%) was calculated as a percent ratio of the actual ethanol produced with respect to the ethanol expected from the total glucose available after acid hydrolysis (Eq. 3).

$$Y_{et} = \frac{\text{Ethanol produced (g)}}{m_{glu} \times 0.51} * 100\% \quad (3)$$

where $Y_{et}$ = ethanol yield; $m_{glu}$ = amount of glucose available after acid hydrolysis (g).

#### 2.4.6 Mass balance calculation

Mass balance of dry matter, carbohydrates, and lignin was calculated using Eqs. (4), (5), (6) and (7), as described by Cybulska et al. [33].

$$\text{Component "i" introduced in the process (g)} = W_{ib} * C_i \quad (4)$$

where $W_{ib}$ = mass of the initial biomass fed into the pretreatment (g); $C_i$ = content of the specific component "i" (dry matter, carbohydrates, and lignin) in the initial biomass fed into the pretreatment (g/gFM).

$$\text{Component "i" recovered after the process (g)} = \frac{W_f * C_f}{100\%} \quad (5)$$

were $W_f$ = mass of the fraction recovered after pretreatment (g); $C_f$ = content of the specific component "i" (dry matter, carbohydrates, and lignin) in the fraction recovered after pretreatment (g/gFM).

$$\text{Solid fraction recovery (\%)} = \frac{\text{Solid dry mass out (g)}}{\text{Dry mass in (g)}} \times 100\% \quad (6)$$

$$\text{Liquid fraction recovery (\%)} = \frac{\text{Liquid dry mass out (g)}}{\text{Dry mass in (g)}} \times 100\% \quad (7)$$

### 2.5 Statistical analysis

The data obtained were statistically analyzed with XLSTAT software (Version 2016.02.2). The Tukey and one-way analysis of variance (ANOVA) tests were used for the comparison of ethanol yields from hydrothermally pretreated RBB. Statistical differences were measured at 95% confidence level ($p < 0.05$).

## 3 Results and discussion

### 3.1 Raw material characterization

The chemical composition of residual banana bulbs (RBB) used in this study is summarized in Table 1. RBB is made of a high amount of carbohydrates, especially glucan. The high glucan content is certainly both from starch and cellulose. RBB has also a low lignin content, suggesting a high enzymatic digestibility potential, as the enzymatic digestibility is known to be negatively affected by high lignin content [36].

### 3.2 Simultaneous saccharification and fermentation of RBB

#### 3.2.1 Ethanol yield after SSF of RBB

Simultaneous saccharification and fermentation (SSF) was directly performed on residual banana bulbs (RBB) to assess their potential as starch and cellulose-based bioethanol feedstock. Based on the characterization of RBB (Table 1), SSF was performed with cellulase, with amylase, and with the

**Table 1** Chemical composition of residual banana bulbs (RBB)

| Component | Content (g/100 gDM RBB) |
|---|---|
| Glucan | 59.62 ± 0.18 |
| Xylan | 4.28 ± 0.06 |
| Arabinan | 2.43 ± 0.04 |
| Klason Lignin | 6.74 ± 0.33 |
| Total Ash | 11.19 ± 0.13 |
| *Water extractives* | *14.06 ± 0.15* |
| *Ethanol extractives* | *1.20 ± 0.03* |
| Total extractives | 15.26 ± 0.15 |
| Residue (not identified) | 0.48 ± 0.43 |
| Total | 100 |

mixture of amylase and cellulase (Novozymes A/S, Denmark) to investigate optimal hydrolysis. Figure 1a shows the ethanol yield in percentage of theoretical ethanol yield after SSF of RBB performed with enzyme mix (amylase and cellulase) and individual enzyme (amylase or cellulase). The weight loss (caused by $CO_2$ released) of the fermentation flasks during SSF is shown in Fig. 1b. The ethanol yield obtained with enzyme mix was higher than that obtained in the absence of enzymes or with individual enzymes, suggesting that both starch and cellulose contribute to the release of fermentable carbohydrates. The maximum ethanol concentration in fermented broth from RBB + enzyme mix was 31 g ethanol/l fermented broth and corresponded to 93% of the theoretical yield based on total available glucose (Fig. 1a). However, the ethanol yield obtained with enzyme mix was lower than the sum of ethanol yield obtained with individual enzymes. The ethanol concentration obtained (31 g ethanol/l fermented_broth) is too low to significantly inhibit *S. cerevisiae* and does not explain why ethanol production is limited after the enzyme mix hydrolysis. The total hydrolytic activity introduced in the fermentation broth would theoretically be able to hydrolyze 6.4 g cellulose and 3.3 g starch in the 24-h pre-hydrolysis step (while neglecting the hydrolysis that can continue during the 5–7 days of fermentation), as compared to the 5 gDM RBB that contained about 0.3 g cellulose and 2.3 g starch ((Awedem et al. (unpublished results)). Incomplete hydrolysis then suggests insufficient access of the enzymes to the target polysaccharides, enzyme inactivation in the medium or some inhibition of the enzymatic activity. Hydrolysis of polysaccharide by one of the enzyme might be inhibited by the accumulation of glucose during the pre-hydrolysis step, as a result of the hydrolysis realized by the other enzyme. However, the inhibition is expected to be released as the glucose fermentation starts and not affect the final result, unless enzymes are losing activity before completion of the substrate hydrolysis. This is supported by the results of the test of enzyme loading (see below), which show that enzymes are a limiting factor. The relative high ethanol yield after cellulase hydrolysis (59% expressed on total glucose, Fig. 1a) is unexpectedly high, when considering our preliminary results that concluded that structural polysaccharides, including cellulose, are less than 20% of the RBB dry matter. This would support the hypothesis that Cellic CTec2 is active in the hydrolysis of other components than cellulose alone. Indeed, Cellic CTec2 is known to be a blend of cellulase, β-glucosidases, and hemicellulases (Novozymes A/S).

In general, the ethanol yield obtained from RBB was higher than that of some similar starch and cellulose-based feedstock like cassava pulp, cassava peel, and starch [7, 23].

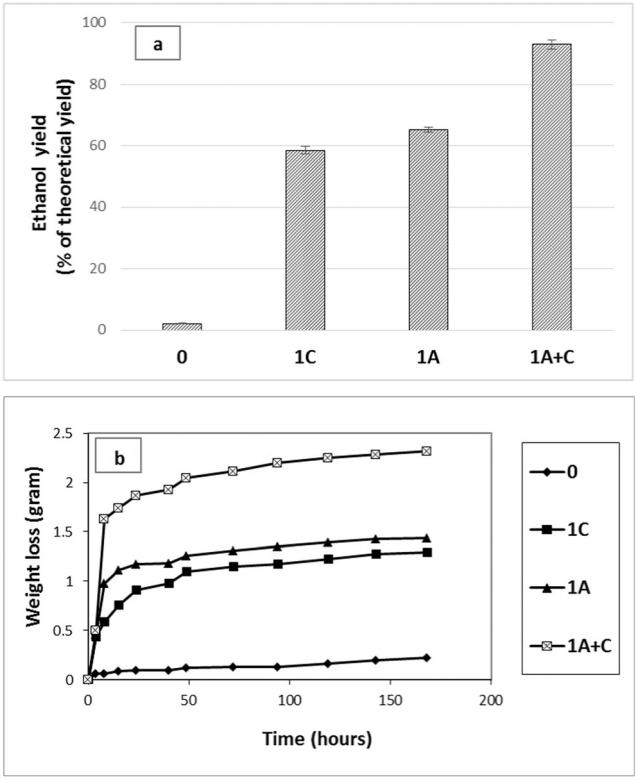


**Fig. 1** **a** Ethanol yield after simultaneous saccharification and fermentation (SSF) of residual banana bulbs (RBB) performed without enzyme (0), with cellulase only (1C), with amylase only (1A), and with cellulase + amylase (1A + C). **b** Weight loss (caused by CO2 released) of the fermentation flasks during SSF

### 3.2.2 Effect of enzyme loading on ethanol yield

Different enzyme loadings starting from the manufacturer (Novozymes A/S, Denmark) recommendation loading range and dilution of it were assessed. Figure 2 shows the effects of enzyme loadings on ethanol yield after SSF of RBB. The reduction of enzyme loading also decreased the ethanol yield. The best ethanol yield (93%) was achieved with the manufacturer recommended enzyme loading ("C": 1 ml of cellulase (activity: 74 FPU/ml_cellulase) + 1 ml of amylase (activity: 14 μmol alpha_1_4_glucose_bond_hydrolyzed/min/ml amylase)). The results confirm that the enzyme dose would be a limiting factor in the SSF of RBB to ethanol, as discussed above.

### 3.2.3 Effect of RBB concentration on hydrolysis and ethanol yield

From the SSF experiment performed with amylase only, it had been visually observed a poor liquefaction and a difficulty to stir, probably related to the high dry matter (DM) content of the RBB (100 gDM/l mixed liquor). In order to examine the influence of DM and starch contents and mixing problems on the results, SSF was performed at lower RBB concentrations. The reduction of RBB concentration reduced viscosity and improved mixing. The obtained ethanol yields are presented in Fig. 3. There was an increase of ethanol yield with RBB at

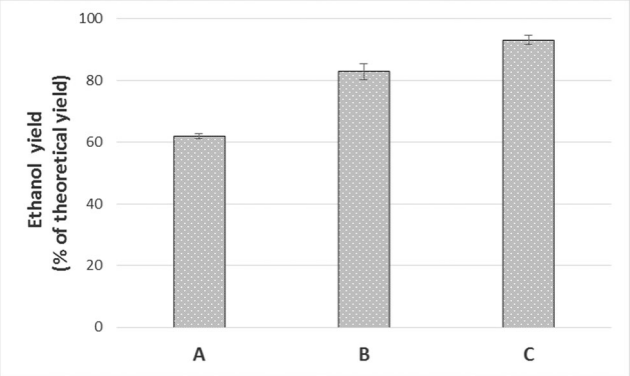


**Fig. 2** Effect of enzyme loading reduction on simultaneous saccharification and fermentation. **a**, **b**, **c** 50%, 75%, and 100% of the cellulase + amylase loading recommended by the manufacturer, respectively

lower concentrations. Concentration "1/4 RBB" gave the highest ethanol yield (Fig. 3). The increase in ethanol yield with lower RBB concentration substrates was then probably due to a better hydrolysis of the substrate, as a result of a better distribution of amylase owing to a more efficient stirring, which released more fermentable carbohydrates. SSF performed with amylase only should therefore be performed with enough water to ensure a good stirring and consequently a good distribution of enzyme. These effects of amylase/RBB concentration are in accordance with the highest ethanol yield (91% of theoretical yield) obtained from the fermentation of cassava pulp at the concentration of 50 gDM/l mixed liquor [7]. An increase of ethanol yield was also observed with RBB at lower concentrations in the absence of amylase, suggesting a better natural availability of fermentable carbohydrates from RBB at that lower concentration and better mixing conditions.

## 3.3 Hydrothermal pretreatment of RBB

The results obtained from the SSF of RBB have shown that the enzymatic hydrolysis was not complete even with the combined use of both amylase and cellulase (Fig. 1a). With the hope to increase the efficiency of both enzymatic hydrolysis and ethanolic fermentation, RBB was submitted to hydrothermal pretreatment.

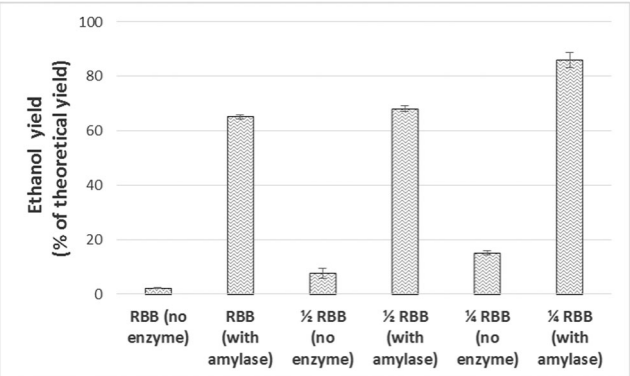


**Fig. 3** Effect of RBB concentration on the hydrolysis by amylase and further fermentation. RBB, 1/2 RBB, and 1/4 RBB: 100, 50, and 25 gDM RBB/l fermentation broths

### 3.3.1 Mass balance of hydrothermal pretreatment

After hydrothermal pretreatment of RBB, the recovered liquid and solid fractions were characterized, and the mass balances were calculated based on the recoveries of the pretreated and initial (RBB) materials. Figure 4 shows the mass balance of the pretreatment process tracking the distribution of dry matter (a), glucose (b), xylose (c), lignin (d), and ash (e) in the residual solid and liquid fractions. Except the ash recovery that was 100% (as compared to ash in initial RBB), the recoveries were lower than 100% for all tracked components. The recoveries were found to be pretreatment temperature depended. Hydrothermal pretreatment induced increasing losses of DM with temperature, ranging from 5% at 100 °C to 16% at 120 °C (Fig. 4a). The absence of ash loss validates that no loss can be attributed to sample handling (Fig. 4e). The reduction in DM mass might then be attributed to the loss of some volatile or semi-volatile compounds or degradation products (e.g., alcohols, aldehydes, ketones) with increasing temperature.

Ashes were more and more solubilized and recovered in the liquid fractions as temperature increased (Fig. 4e). However, glucose and xylose that disappeared from the solid fraction were not significantly recovered in the liquid fraction (Fig. 4b, c), neither as polysaccharide nor as monosaccharide. Lignin followed the same trend (Fig. 4d).

### 3.3.2 Effect of hydrothermal pretreatment on ethanol yield

Figure 5 shows the ethanol yield after SSF of the solid fractions recovered after hydrothermal pretreatment and RBB performed with enzyme mix (amylase and cellulase). RBB gave the higher conversion to ethanol, as compared to all pretreated materials. The maximum ethanol yield in fermented broth of RBB was 31 g ethanol/l mixed liquor and corresponded to 93% of theoretical yield based on available glucose (Fig. 5). The maximum ethanol yields (based on available glucose) achieved with pretreated samples ranged from 65 to 81% and suggested low glucose convertibility as compared to initial RBB. No significant statistical differences ($p < 0.05$) were observed between the ethanol yields obtained from RBB pretreated at 100, 110, and 120 °C. The lower ethanol yields observed with solid fractions recovered after hydrothermal pretreatment indicate that the residual glucose was less fermentable than in the initial RBB, probably because it was in the form of crystalline cellulose or due to the presence of fermentation inhibitors. In the temperature

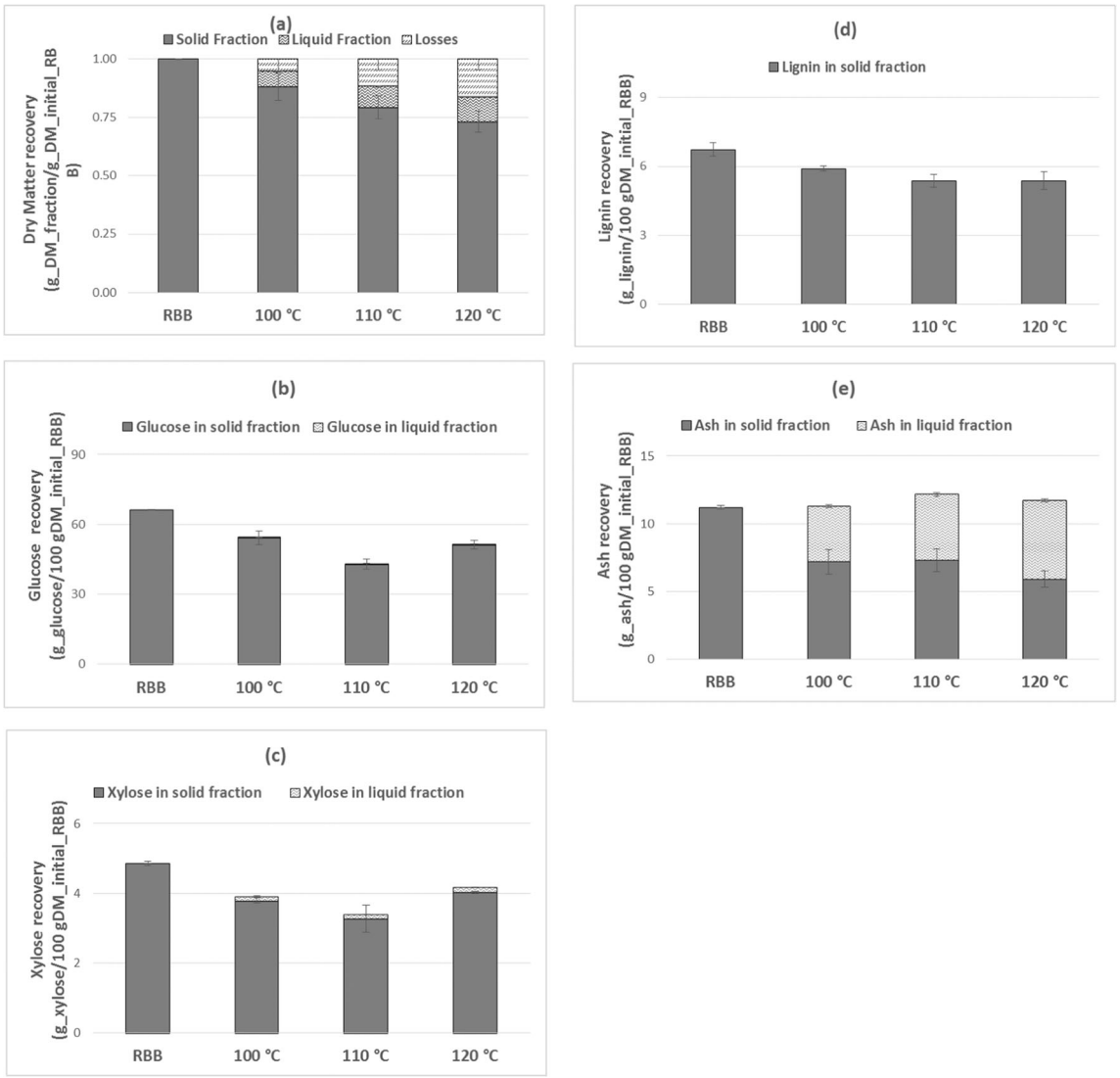


**Fig. 4** Mass balance of the hydrothermal pretreatment process tracking the recovery of dry matter (**a**), glucose (**b**), xylose (**c**), lignin (**d**), and ash (**e**). RBB: residual banana bulbs; 100 °C, 110 °C, and 120 °C: liquid and solid fractions recovered after hydrothermal pretreatment of RBB at 100 °C, 110 °C, and 120 °C

range tested, Fig. 5 clearly shows that hydrothermal pretreatment did not enhance the ethanol yields of the solid fractions. Therefore, banana bulbs biomass should better be used for bioethanol production without any thermal pretreatment process.

To check the presence of potentially inhibitory substances (furfurals, acids) in the liquid fractions recovered after hydrothermal pretreatment, the SSF of the solid fractions was performed using the liquid fractions as fermentation medium. Figure 5 compares the ethanol yield after SSF of solid fractions using liquid fraction and water as fermentation medium. The ethanol yield obtained from the SSF performed with liquid fractions recovered after hydrothermal pretreatment as fermentation medium were always slightly lower than the ethanol yield obtained from the SSF performed with water as fermentation medium (Fig. 5). However, except the SSF performed with liquid fraction of RBB pretreated at 120 °C, no statistical significant difference ($p < 0.05$) was observed between the ethanol yields obtained from the SSF performed with water and the ethanol yields obtained from the SSF performed with liquid fractions as fermentation medium (Fig. 5).

Table 2 shows the concentration of by-products and inhibitors in the liquid fractions recovered after pretreatment. The concentration of acetic acid, succinic acid, and malic acid increased with the increase of pretreatment temperature. Acetic acid was the major organic acid measured. As expected, the furfural content also increased with increased temperature and ranged from 0.10 to 0.23 mg/l liquid fraction for samples pretreated at 100 to 120 °C, respectively. HMF (5-hydroxymethylfurfural) was below the detection limit (25 μg/l liquid fraction). It is known that xylose and glucose dissolved

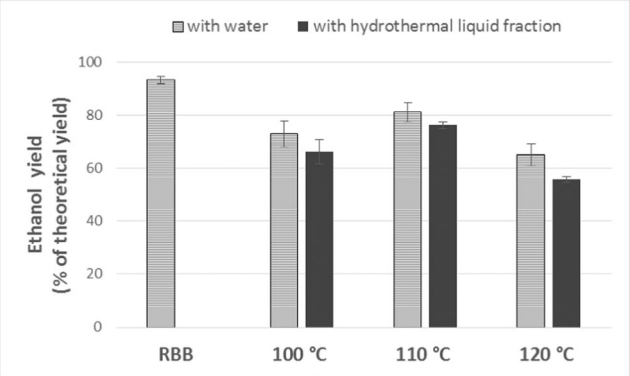


**Fig. 5** Ethanol yield after simultaneous saccharification and fermentation of residual banana bulbs (RBB) and solid fractions recovered after pretreatment performed with cellulase + amylase, and using either water (lined bars) or hydrothermal liquid fractions (black bars) as fermentation medium (both fermentation medium acidified to pH 4.8 with sulfuric acid). 100 °C, 110 °C, and 120 °C: solid and liquid fractions recovered after pretreatment of RBB at 100 °C, 110 °C, and 120 °C

into liquid fraction during pretreatment are degraded to furfural and HMF, respectively [10]. The low amount of furfurals recovered are far below the amounts corresponding to the glucose and xylose disappeared during the pretreatment, but could possibly explain the decline of the ethanol yields observed with SSF using the liquid fractions as fermentation medium (Fig. 5). However, considering the low total amount of pretreatment by-products and inhibitors recovered in the liquid fractions, liquid fractions could still be used as fermentation medium to reduce water consumption of the process, as it allowed to achieve 56 to 78% of theoretical ethanol yield, as compared to 65 to 81% of theoretical ethanol yield obtained with SSF using water (Fig. 5). Nevertheless, the results obtained with pretreated materials were consistent with those published by Akihiko et al. [7] who reported a lower ethanol yield from cassava pulp pretreated in the same temperature range, as compared to non-pretreated cassava pulp.

### 3.4 Renewable energy potential of bioethanol from banana bulbs biomass: case study of Cameroon

Table 3 presents an assessment of the energy that could be generated as bioethanol from residual banana bulbs biomass in Cameroon. This assessment was restricted to the biomass of the Grande Naine (GN) variety, which is the commercially most produced variety in Cameroon. The locally used plantain has not been considered (lack of data on available biomass). Only bioethanol production from the (non-treated) banana bulbs (RBB) was considered. Table 3 shows that the amount of banana bulbs residues available annually in Cameroon for the sole variety GN is significant. Bioethanol potential of this biomass has been estimated at 400 l/tons DM RBB (or 310 kg/tons DM_RBB). This bioethanol potential is comparable to the bioethanol potential of corn (400 l/tons_DM), which is one of the most used feedstock for ethanol production in the world [5, 6, 39].

When converted to energy, the bioethanol produced could supply an annually estimated 79.9 GWh of energy (Table 3). This is the crude energy content of the produced ethanol, while not subtracting the energy needed by the production process. The energy needed by the process (electricity and heat) could be supplied in the renewable form from the anaerobic digestion of the other banana residues as described by Awedem et al. [30, 31]. The annual Cameroon motor gasoline consumption in 2012 was about 427,285 ton oil equivalents (toe) and corresponded to 4968 GWh/year [38]. If we take this value into account, the residual banana bulbs biomass could cover about 1.6% of the annual Cameroon motor gasoline consumption. The RBB contribution of 1.6% of the transportation requirement of Cameroon may seem small, but this corresponds to about 6880 toe/year, which is certainly more than the amount of fuel consumed in plantations by tractor with trailer during the transportation of RBB from the fields to the bioethanol production plant. Moreover, if we take into account the other banana varieties cropped in Cameroon for local needs, this renewable energy contribution could be doubled [3, 40].

Nevertheless, this simplified assessment does not take into account all the practical constraints (e.g., bulbs harvest and processing, cost of enzymes, ethanol valorization, etc.) of the implementation of a bioethanol production plant. It allows, however, to set the orders of magnitude of the energy potential. The energy derived from bioethanol could contribute to an environmentally and economically sustainable development of the country.

**Table 2** Concentration of the by-products and inhibitors detected in the liquid fraction (mg/l LF)

| | Acetic acid | Succinic acid | Malic acid | Furfural | HMF |
|---|---|---|---|---|---|
| 100 °C | 52.38 ± 0.33 | 24.15 ± 0.44 | 19.02 ± 1.59 | 0.10 ± 0.00 | n.d |
| 110 °C | 67.46 ± 2.38 | 28.29 ± 0.77 | 32.05 ± 2.80 | 0.15 ± 0.00 | n.d |
| 120 °C | 96.10 ± 3.79 | 46.55 ± 0.80 | 40.58 ± 4.81 | 0.23 ± 0.00 | n.d |

*LF* liquid fraction, *HMF* 5-hydroxymethylfurfural, *n.d* not detected at 25 μg/l LF

**Table 3** Estimation of the renewable energy that could be generated annually as bioethanol in Cameroon from residual banana bulbs of the commercially most produced Grande Naine variety

| | |
|---|---|
| Annual production of fruits (ton_FM/year)[a] | $1.4 \times 10^6$ |
| Annual fresh residual biomass production (ton_FM/year)[b] | $2.8 \times 10^6$ |
| Annual dry residual biomass production (ton_DM/year)[b] | 313,600 |
| Distribution of banana bulb fraction in banana residual biomass (%_DM)[c] | 11 |
| Annual biomass production of banana bulb fraction (ton_DM/year) | 34,496 |
| Bioethanol potential of banana bulbs (kg_EtOH/ton_DM) | 310[f] |
| Bioethanol potential of banana bulbs (kg_EtOH/year) | $11 \times 10^6$ |
| Bioethanol potential of banana bulbs (liter_EtOH/year) | $14 \times 10^6$ |
| Energy in bioethanol derived from banana bulbs residues (GWh_EtOH/year)[d] | 79.9 |
| Annual transportation requirement of Cameroon (GWh/year)[e] | 4968 |

[a] Obtained from the annual production of bananas in Cameroon [4]

[b] Estimated with 11.2% DM of annual production [1, 3]

[c] Obtained from Kamdem et al. [3, 37]

[d] Calculated from the lower heating value (LHV) of 1 kg of EtOH (7.47 KWh/kg) [3]

[e] Obtained from EIA (2015) cited by [38]

[f] Fig. 1a

## 4 Conclusion

Residual banana bulbs (RBB) showed a good potential as a bioethanol feedstock. Both cellulase and amylase are needed to efficiently hydrolyze RBB. The highest ethanol yield (93% of theoretical production based on glucose) was obtained with RBB and corresponded to 310 kg EtOH/ton_DM_RBB. Hydrothermal pretreatment of RBB affected negatively their conversion to ethanol. When considering the RBB resulting from the Grande Naine variety cropped in Cameroon, the energy derived from bioethanol is about 80 GWh EtOH/year and corresponds to 1.6% of the annual transportation requirement of Cameroon. This energy could contribute to meet the energy requirements of human activities in the tropical banana-producing countries.

**Acknowledgments** They are also grateful to Novozymes A/S (Denmark) for the gift of enzymes. A great acknowledgment to Kathrine Hansen (Department of Energy Technology, Aalborg University, Esbjerg, Denmark) and Linda Madsen (Department of Chemical Engineering, Aalborg University, Esbjerg, Denmark) for their technical assistance. M.D. is Senior Research Associate of the F.R.S.-F.N.R.S. (National Funds for Scientific Research, Belgium).

**Funding information** This work was financially supported by the Wallonia-Brussels International (WBI) and the Ministry of Higher Education, Research and Media of Wallonia-Brussels Federation (FWB) from Belgium.



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