Original Article

WM&R

Waste Management & Research
2015, Vol. 33(11) 1022–1032

DOI: 10.1177/0734242X15597998
wmr.sagepub.com
SAGE

Comparative biochemical analysis after steam pretreatment of lignocellulosic agricultural waste biomass from Williams Cavendish banana plant (Triploid *Musa* AAA group)

Irénée Kamdem[1], Nicolas Jacquet[2], Florian Mathias Tiappi[2], Serge Hiligsmann[1], Caroline Vanderghem[2], Aurore Richel[2], Philippe Jacques[1] and Philippe Thonart[1]

Abstract

The accessibility of fermentable substrates to enzymes is a limiting factor for the efficient bioconversion of agricultural wastes in the context of sustainable development. This paper presents the results of a biochemical analysis performed on six combined morphological parts of Williams Cavendish Lignocellulosic Biomass (WCLB) after steam cracking (SC) and steam explosion (SE) pretreatments. Solid (S) and liquid (L) fractions (Fs) obtained from SC pretreatment performed at 180°C (SLFSC180) and 210°C (SLFSC210) generated, after diluted acid hydrolysis, the highest proportions of neutral sugar (NS) contents, specifically 52.82 ± 3.51 and 49.78 ± 1.39 %w/w WCLB dry matter (DM), respectively. The highest proportions of glucose were found in SFSC210 (53.56 ± 1.33 %w/w DM) and SFSC180 (44.47 ± 0.00 %w/w DM), while the lowest was found in unpretreated WCLB (22.70 ± 0.71 %w/w DM). Total NS content assessed in each LF immediately after SC and SE pretreatments was less than 2 %w/w of the LF DM, thus revealing minor acid autohydrolysis consequently leading to minor NS production during the steam pretreatment. WCLB subjected to SC at 210°C (SC210) generated up to 2.7-fold bioaccessible glucan and xylan. SC and SE pretreatments showed potential for the deconstruction of WCLB (delignification, depolymerization, decrystallization and deacetylation), enhancing its enzymatic hydrolysis. The concentrations of enzymatic inhibitors, such as 2-furfuraldehyde and 5-(hydroxymethyl)furfural from LFSC210, were the highest (41 and 21 μg ml^{-1}, respectively). This study shows that steam pretreatments in general and SC210 in particular are required for efficient bioconversion of WCLB. Yet, biotransformation through biochemical processes (e.g., anaerobic digestion) must be performed to assess the efficiency of these pretreatments.

Keywords

Biochemical analysis, enzymatic inhibitors, lignocellulosic agricultural waste biomass, polysaccharides acid hydrolysis, steam explosion, steam pretreatment, sustainable development, Williams Cavendish banana plant

Introduction

Banana and plantain plants (*Musa spp*.), which will be referred to as banana plants in this paper, are giant herbaceous plants that are perennial and monocarpic and range from 2 to 15 m in height. The fruit of banana plants is the world's fourth most important agricultural product after rice, wheat and maize (FAO, 2013; Lassoudière, 2007, 2012). In terms of commercialization, banana is the most exported fruit in terms of both value and quantity (Lassois et al., 2009). The interest in banana plants is increasing with the exponential growth (approximately 80 million per year) of the world's population (Fischer and Heilig, 1997). The importance and multiple uses of these plants encourage the study of their genetics and classification (Carreel et al., 2002; Global Musa Genomic Consortium, 2001; Shepherd, 1999; Simmonds and Shepherd, 1955), culture and trade (Honfo et al., 2011; INIBAP, 1999; Lassoudière, 2007, 2012) and chemical composition and biorefinery (Happi et al., 2008, Kamdem et al., 2011; Oliveira et al., 2007). Yet, banana producers, particularly agro-industrial companies that produce bananas, generate a significant quantity of lignocellulosic wastes. These companies are facing

[1]Walloon Centre of Industrial Biology (CWBI), University of Liege, Belgium
[2]Unité de chimie biologique industrielle, University of Liege, Gembloux Agro-Bio Tech, Belgium

Corresponding author:
Irénée Kamdem, Walloon Centre of Industrial Biology (CWBI), University of Liege, Bd du Rectorat, 29, B40–P70, 4000 Liege, Belgium.
Email: kamire88@gmail.com or ikamdem@doct.ulg.ac.be

many challenges that prevent them from potentially ameliorating their income and contributing efficiently to the reduction of greenhouse gases (mostly produced by the combustion of fossil fuels). These challenges are centred on the efficient valorization of banana lignocellulosic biomass (BLB), which is mostly composed of six morphological parts (MPs) that are bulbs, leaf sheaths, petioles-midribs, leaf blades, rachis stems and floral stalks (Kamdem et al., 2013). These abundant biological resources consist of molecules or chemical elements with high energy and economic potential. Biopolymers such as cellulose (26.09 %w/w), lignin (18.36 %w/w), hemicelluloses (10.89 %w/w), pectin (7.73 %w/w), starch (5.63 %w/w), proteins (4.94 %w/w) and lipids (5.26 %w/w of BLB's dry matter (DM)) are the main macromolecules of BLB (Kamdem et al., 2011; Oliveira et al., 2007).

Under the global threat of fossil fuels, the massive presence and increasing expansion of banana plantations require the optimal energetic conversion of BLB abandoned on plantations after fruit harvesting. Considering the chemical composition of BLB (Kamdem et al., 2011; Lassoudière, 2007, 2012; Oliveira et al., 2007), energy vector production (such as biomethane, biohydrogen and bioethanol) through the biochemical conversion of this biomaterial appears to be the most suitable process for energy conversion (Demirbas, 2011; Hiligsmann et al., 2011; Laurent et al., 2011; Wertz, 2012). It is well known that accession of enzymes to fermentable macromolecules is an energetic bioconversion limiting factor. Therefore, pretreatment of this reported lignocellulosic biomaterial must be performed to optimize the accession of these fermentable macromolecules to enzymes. The anaerobic digestion of BLB reported by Kamdem et al. (2013) has demonstrated that this substrate requires pretreatment to optimize energy vector production through the bioconversion process.

The aim of the pretreatments is firstly to delignify the biomaterial and secondly to modify physical and physicochemical properties, such as the degree of polymerization and state of crystallinity of the cellulose fraction (Didderen et al., 2008; Janga et al., 2012; Ogier et al., 1999). Among pretreatments required to optimize the accessibility of BLB-derived fermentable substrates to enzymes, physicochemical treatments, that are, steam cracking (SC) and steam explosion (SE), are among the most suitable processes because of their economic, environmental, energy consumption and technological advantages (Brownell and Saddler, 1987; Didderen et al., 2008; Jacquet, 2012; Jacquet et al., 2012; Ogier et al., 1999; Wertz, 2012). To our best scientific knowledge, no study concerning the energetic valorization of the combined six MPs of BLB has been published. Optimal energy vector production from this biomaterial therefore implies its chemical deconstruction through economically advantageous and efficient pretreatments.

SE is composed of two distinct phases: the SC or thermohydrolysis phase (at high pressure), and the explosive decompression phase. This process helps to deconstruct lignocellulosic material by combining SC hydrolysis action induced by the formation of organic acids (such as acetic and uronic acids) and shear action resulting from the sudden drop in pressure (Ramos et al., 1992).

The efficiency of pretreatments prior to enzymatic digestion is closely related to operating conditions (Carvalheiro et al., 2008; Clark et al., 1989; Jacquet et al., 2011; Overend et al., 1987). Studies examining steam pretreatments indicate that relationships can be established between the temperature of the process, the retention time, the time needed (i.e., depending on the volume of the reactor) to reach the target pressure and the accessibility of the substrate, resulting in the improvement of hydrolysis yields. Therefore, the bioconversion yield of lignocellulosic biomass after physicochemical pretreatment depends on the treatment severity factor, which is defined as a value highlighting the intensity of the pretreatment (Jacquet et al., 2011). This factor can be used to describe and compare the efficiency of steam pretreatments on lignocellulosic substrates. Formation of fermentation inhibitors such as furans (5-HMF and 2F), phenols (*p*-coumaryl, coniferyl and synapyl alcohols) and carboxylic acids (acetic and uronic acids) during physicochemical pretreatment of cellulosic polymer and lignocellulosic materials have been reported by Jacquet et al. (2012), Klinke et al. (2004) and Pienkos and Zhang (2009).

To determine the optimal pretreatment conditions or severity factors for BLB energetic transformation through biochemical processes, SC and SE pretreatments under different experimental conditions or with different severity factors (selected for their relatively good performance for lignocellulosic biomass deconstruction) were examined in the present study. The experiments were performed with lignocellulosic material from six combined MPs of Williams Cavendish banana plant (Triploid *Musa* AAA group) cultivated in Cameroon. Hydrolysis of the treated materials was performed, and biochemical analysis was carried out to assess pretreatment efficiency.

Materials and methods

Sample collection and preparation

Approximately 100 kg (moisture of approximately 90 %w/w) of Williams Cavendish Lignocellulosic Biomass (WCLB) was randomly collected from an agro-industrial banana plantation (Cameroon Development Corporation (CDC)-Del Monte) in Moussaka (village situated in the southwest of Cameroon) after the mature fruits had been harvested by cutlass. WCLB was then carefully separated into six different MPs: bulbs, leaf sheaths, petioles-midribs, leaf blades, rachis stems and floral stalks. These MPs were cut into pieces with diameters of approximately 50 mm and were then washed, rinsed four times to remove pesticide residues and finally sun and air-dried for 30 days, as described by Kamdem et al. (2013). To conduct efficient and reproducible experiments, homogeneous material representing the entire WCLB was constituted at the laboratory and pilot scales. Approximately 3 kg based on DM of the entire WCLB was reconstituted, as presented in Figure 1. The reconstitution of the lignocellulosic biomass was performed according to the proportions of the different MPs of the plants without the fruits. Prior to experiments, which were carried out in duplicate, the reconstituted biomass was chopped into small pieces approximately 3 mm in diameter. The final DM content was 93 %w/w.

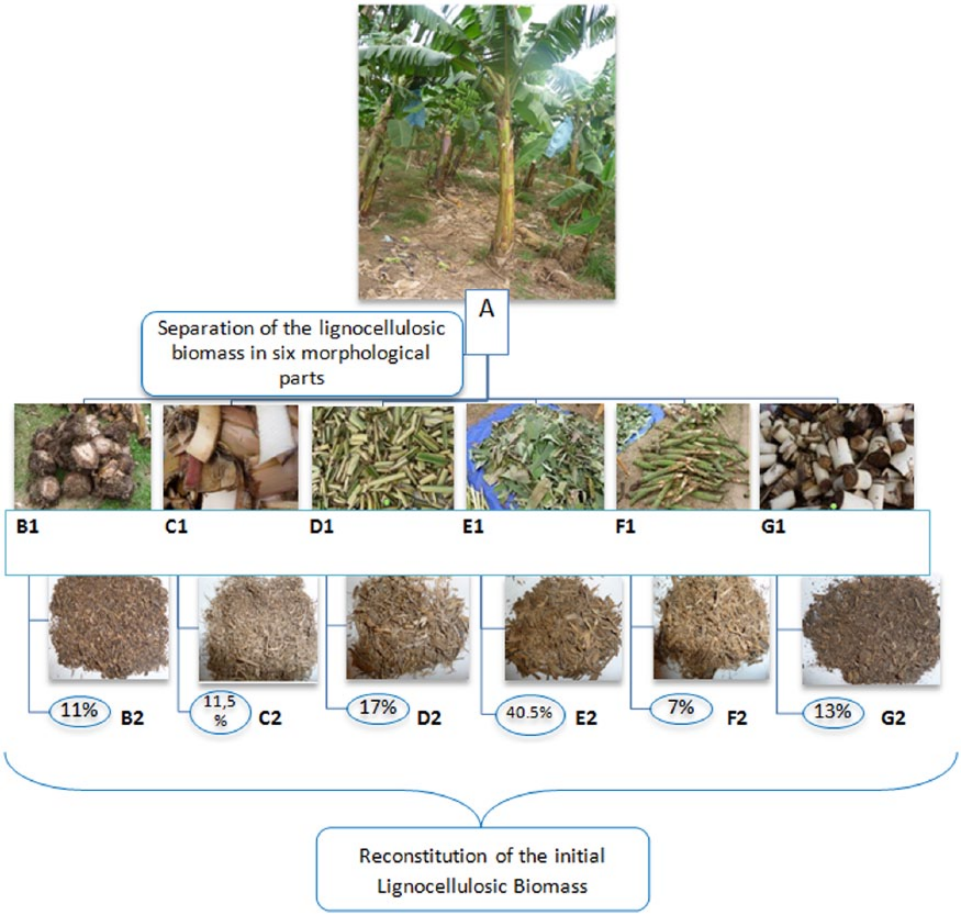

Figure 1. Preparation of Williams Cavendish Lignocellulosic Biomass from six combined morphological parts.
A indicates Fresh Williams Cavendish banana plant on a banana plantation. B1 and B2, C1 and C2, D1 and D2, E1 and E2, F1 and F2, G1 and G2 indicate fresh and sun-dried material from bulbs, leaf sheaths, petioles-midribs, leaf blades, rachis stems and floral stalks, respectively.

Physicochemical pretreatment of WCLB

Pretreatment of WCLB was carried out with physicochemical prototype pilot-scale treatment equipment, designed in the department of Industrial Biological Chemistry-ULG-Gembloux Agro-Bio Tech. The same equipment was used to perform SC and SE, as described by Jacquet et al. (2010). This prototype consists of a steam generator (operating pressure: 6.0 MPa), a 50-l vessel designed to reach a maximum operating pressure of 5.1 MPa and a cyclone explosion tank, where the exploded product is recovered. A quick-opening ball valve, placed between the vessel and the explosion cyclone tank, is used to release and quickly decrease pressure and provide the explosion effect. The severity factor was calculated according to the following integral equation, described by Jacquet (2012), Jacquet et al. (2011) and Li and Chen (2008):

$$S = \mathrm{Log}_{10}\sum \frac{14.75\left(t_{n+1}-t_n\right)}{\left(T_{n+1}-T_n\right)}\left[\frac{\exp\left(\frac{T_{n+1}-100}{14.75}\right)}{\exp\left(\frac{T_n-100}{14.75}\right)}\right].dt \quad (1)$$

where S indicates the severity factor; t_n and t_{n+1}, the initial increment times $_n$ and $_{n+1}$; T_n and T_{n+1}, the process temperatures for times t_n and t_{n+1}, respectively; 100, the boiling point of water; and 14.75, the activation energy value under conditions where process kinetics are of the first order and obey the Arrhenius law.

SC operations were carried out in duplicate using 250 g of WCLB DM under three different experimental conditions, consisting of the process target temperature (T), target pressure (P) and retention time (t). These conditions are represented by the triplet (T, P, t), where T, P and t are expressed, respectively, in °C, MPa and min. After placing the material in the reactor, the triplet (combination of the operating variables) was adjusted to the desired treatment conditions (°C, Mpa and min). As described by Jacquet et al. (2011) and Li and Chen (2008), the (T, P, t) combinations used in this study were (150, 0.4, 3.38), (180, 0.87, 4.12) and (210, 1.75, 5.03), leading, respectively, to severity factors of 2.48, 3.38 and 4.29. These triplets are mostly used for the physicochemical pretreatment of herbaceous lignocellulosic biomass (Han et al., 2010; Jacquet et al., 2011; Li and Chen, 2008). Given that temperature increases linearly with any increment in pressure (0.1 Mpa) (Jacquet et al., 2011), the initial measurement of retention time (duration of the time that the material was subjected to the target temperature) started when the vessel reached 96 % of the target pressure. The time required to reach the different target pressures was approximately 35, 42 and 51 min for 0.4, 0.87 and 1.75 Mpa, respectively.

To prevent material loss and ensure explosive effects due to sudden decompression during the SE process (Ibrahim et al., 2010; Martin-Sampedro et al., 2011), a larger amount of WCLB DM was used to perform SE pretreatment in duplicate (500 g). The triplet used was (210, 1.75, 3), and the demand time was approximately 51 min. The calculated severity factor was 3.16.

Pretreated materials were then separated into a black liquor or liquid fraction (LF) and a solid fraction (SF), as described by Jacquet et al. (2011). The liquid and SFs was stored at −20°C and used for further investigations. SFs were washed with distilled water before chemical characterization.

Analytical methods

Expression of data. Analytical determinations were performed in duplicate. Values were expressed as the mean ± average deviation (mean ± ADv) where the presentation of the results required it.

Chemical analysis of WCLB.

Dry matter content. Approximately 0.5 g of ground WCLB was oven-dried at 105°C to a constant weight. The DM content was determined according to the following equation:

$$DM = \left(DW / IW\right) \times 100 \quad (2)$$

where "*DW*" is the dry weight obtained after oven drying and "*IW*" is the initial weight of the ground matter before oven drying.

Determination of furfural and 5-(hydroxymethyl)furfural. The method used in this study was obtained from Jacquet et al. (2011) with some modification. Approximately 5 ml of the LF derived from pretreated WCLB was filtered through a 0.45 µm filter, and 20 µl of the filtrate was injected into a Waters high performance liquid chromatography (HPLC) system (Waters 2695, Waters Corporation, Milford, USA) equipped with a diode array detector (Waters 2690 Separation Module, Waters 996 Photodiode Array Detector). The HPLC column was an Agilent Zorbax 300SB-C18 4.6x150 mm, 5 m. The column temperature was 30°C. Solvent A was 90% water (with 1% acetic acid) and 10% methanol, and solvent B was CH_3CN (HPLC grade solvents). The gradient was as follows: 0–5' (0% of B), 5–10' (linear gradient up to 100% of B), 10–15' (100% of B), 15–20' (linear gradient up to 0% of B), 20–30' (0% of B). As described by Jacquet et al. (2011), calibration was performed with four solutions containing 2-furaldehyde (2F, Acrosorganic, ref. 181100250) and 5-(hydroxy-methyl)-2-furaldehyde (5-HMF, Acrosorganic, ref. 121460010); the concentrations of the 2F and 5-HMFs solutions were 0.8, 4.4, 22 and 110 g ml^{-1} H_2O.

Water and ethanol extractives contents. To prevent interference in later analytical steps, it is necessary to remove non-structural material from biomass prior to analysis. Generally, the extraction procedure uses a two-step process to remove water-soluble and ethanol-soluble material. According to Sluiter et al. (2005), water soluble materials may include inorganic material, non-structural sugars and nitrogenous material, among others. Ethanol soluble material includes chlorophyll, waxes and other minor components. To determine extractive contents, samples were successively extracted with hot water (100°C) and hot ethanol (960 ml l^{-1}) in a Soxhlet extractor (Sluiter et al., 2005). Approximately 1 g of SFs (DM) of treated WCLB and raw WCLB (RWCLB) were subjected to extraction. After extraction, the remaining material was air-dried under ventilation at 50°C and used to determine the lignin and monosaccharide contents.

Insoluble and soluble lignin contents. Acid-insoluble and acid-soluble lignin contents were determined as described by Sluiter et al. (2008) and Ehrman T (1996), respectively. Approximately 300 mg (DM) of the previous materials subjected to extractions (SFs of treated WCLB and RWCLB) were used to determined acid-insoluble lignin content by the gravimetric method. Acid-soluble lignin was measured spectrophotometrically by reading the ultraviolet (UV) absorbance at 205 nm in the hydrolysate obtained after filtration of the cooled insoluble lignin. After correction to an as-received basis, total lignin content in the sample was the sum of the insoluble and acid lignin.

Monosaccharide composition. Monosaccharide composition in the SFs was determined after 1 h of prehydrolysis of the samples in 72% sulphuric acid at 30°C followed by 3 h of hydrolysis at 100°C in 1 M sulphuric acid. LFs were not hydrolysed. Monosaccharides were determined as alditol acetates by gas chromatography (GC). Reduction of monosaccharides and acetylation was performed according to the procedure described by Blakeney et al. (1983). Analyses were carried out with a Hewlett-Packard gas chromatograph (HP 6890, Agilent Technologies Inc., Hewlett-Packard Inc. USA) equipped with a flame ionization detector, as described by Vanderghem et al. (2012). Data were analysed using ChemHP software. 2-Deoxyglucose (internal standard), glucose, xylose, arabinose, mannose, galactose and rhamnose were obtained from SigmaAldrich (St. Louis, USA). Solutions of known concentrations were used as standards. After correction to an as-received basis, the total content of each monosaccharide, expressed as the percentage of WCLB DM, was calculated as follows:

$$M_T = \left(A \times M_{SF}\right) + \left(B \times M_{LF}\right) \quad (3)$$

where "M_T" is the total monosaccharide content, "A" is the proportion of SF, M_{SF} is the monosaccharide content of the SF, "B" is the proportion of LF and "M_{LF}" is the monosaccharide content of the LF.

Enzymatic cellulose degradation. To ensure optimal enzymatic degradation of holocellulose, an enzymatic mixture composed of cellulases and hemicellulases, from *Humicola insolens*, *Trichoderma reesei* and *Aspergillus aculeatus* commercialized as Celluzyme, Celluclast 1.5L, and ViscozymeL, respectively, by Novo Nordisk (Bagsvaerd, Danemark) was used. According to Rodriguez (2005), the combination of exoglucanase, endoglucanase, β-glucanase and xylanase activities from this mixture resulted in better cellulolytic and hemicellulolytic activities than that of a purified analytical cellulase complex from *Trichoderma reseei* (Sigma-Aldrich, St. Louis, USA).

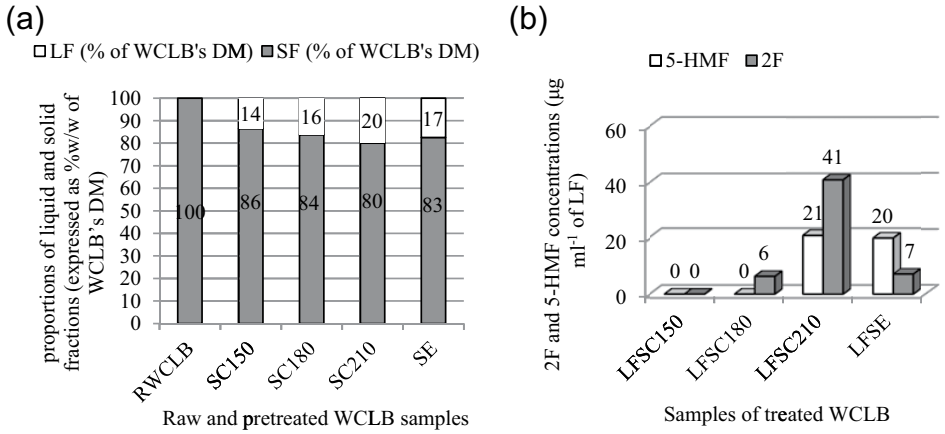

Figure 2. (a) Proportions of liquid and solid fractions from SC and SE pretreated WCLB. (b) 2-Furfuraldehyde (2F) and 5-(hydroxymethyl)furfural (5-HMF) contents of the liquid fractions expressed as µg ml^{-1}.
SC150, SC180 and SC210: steam cracking pretreatment performed at 150, 180 and 210°C, respectively; SE: steam explosion; SC: steam cracking; LF: liquid fraction; SF: solid fraction; DM: dry matter; WCLB: Williams Cavendish Lignocellulosic Biomass; LFSC150, LFSC180 and LFSC210: liquid fractions of 150, 180 and 210°C steam cracked samples, respectively; LFSE: liquid fraction of steam exploded sample; LF: liquid fraction.

Holocellulose (hemicellulose and cellulose) accessibility to cellulase and hemicellulase was assessed using 30 ml of an enzymatic mixture, prepared as described by Rodriguez (2005) and Rodriguez et al. (2005). The mixture comprised 5 volumes of celluzyme (85 and 200 mUI ml^{-1} of FPase and xylanase activity, respectively), 3 volumes of dialysed Viscozyme (62 and 100 mUI ml^{-1} of FPase and xylanase activity, respectively), 1.5 volumes of dialysed Celluclast (176 and 135 mUI ml^{-1} of FPase and xylanase activity, respectively) and 0.5 volumes of KH_2PO_4/Na_2HPO_4 buffer (0.1 M) containing 0.05% NaN_3. The final FPase and xylanase activity of the mixture was 350 and 420 mUI ml^{-1}, respectively. Approximately 1 g (DM) of each fraction from treated WCLB (LF and SF) and RWCLB was hydrolysed at 40°C and pH 5.5 over 48 h of incubation according to the enzymatic cellulose degradation (ECD) method described by Rodriguez et al. (2005). After incubation, the hydrolysates were centrifuged for 10 min at 10,000 g, and the supernatant was filtered through a 0.2-µm membrane (Minisart syringe filter, Vivascience, Hannover, Germany). Monosaccharide contents (glucose and xylose) were then determined by HPLC as described by Rodriguez (2005) and Lechien et al. (2006). Given that xylose represents 80% of sugar content in the hemicellulosic fraction (Chandel et al., 2012), bioaccessible hemicelluloses could then be estimated.

Results and discussion

Pretreatments and furfural content

Pretreatments. After the reconstitution of WCLB (as described previously in the *Sample collection and preparation* section), the samples were subjected to physicochemical pretreatments. The proportions of the resulting fractions are presented in Figure 2(a).

The proportions of the liquid and SFs (expressed as %w/w of WCLB DM) were, respectively, 14 and 86 %w/w for WCLB subjected to SC150, 16 and 84 %w/w (to SC180), 20 and 80 %w/w (to SC210) and 17 and 83 %w/w (to SE). As presented in Figure 2(a), the obtained results from the SC pretreatments indicated that DM distribution in the LFs increased with severity factor, moving from 16 (severity factor of 2.48) to 20 %w/w (severity factor of 4.29) of WCLB DM. As described by Mok and Antal (1992) and Van Walsum et al. (1996), this result could be explained by partial or total solubilization of the biomass components (e.g., ash components or mineral elements, cellulose, hemicelluloses, lignin and pectin) at high temperatures during the process (up to 230°C). These components are structured and organized to form the plant cells that constitute the plant biomass. Thus, the pretreatments cause the disruption and destruction of the cells and thereby allow the relative solubilization of their soluble components and other byproducts formed during the treatment process. This relative solubilization of the components and the byproducts explains the material removal from the initial biomass and therefore justifies the relative distribution of the materials that constitute the DM of the solid and LFs of treated WCLB. Hendriks and Zeemann (2009) also demonstrated that solubility increases with increasing temperature. A decrease in solubility with decreasing severity factor was also observed with the SE sample, where the LF DM was 17 %w/w, confirming that the solubility of WCLB compounds increases with the severity factor.

SF proportions (80 and 83 %w/w of WCLB DM, respectively) obtained with SC and SE pretreatment at 210°C were comparable to the results obtained by Laser et al. (2002) during their study of sugarcane bagasse. In the experiment conducted by Laser et al. (2002), the SF of treated sugarcane bagasse obtained via the SE process at 220°C and a 5 min retention time was higher (at least 65 %w/w) than the SF obtained via the SC process (56 %w/w).

Furfural content. The quantities of the 2F and 5-HMF products in LFs were determined to detect the formation of these fermentation inhibitors during SC and SE pretreatments. The results are

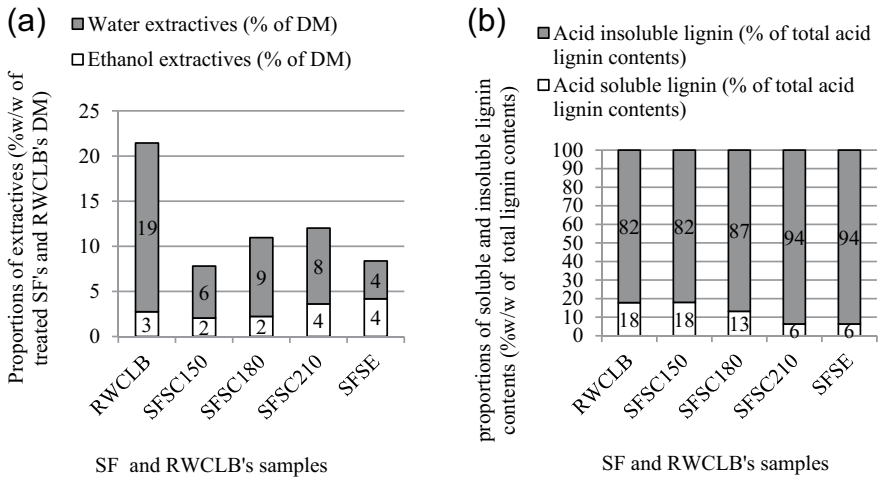

Figure 3. (a) Proportions of water and ethanol extractives expressed as %w/w of SF and RWCLB dry matter. (b) Proportions of acid soluble and insoluble lignin contents expressed as %w/w of total acid lignin contents of SFs and RWCLB.
SFSC150, SFSC180 and SFSC210: solid fractions of 150, 180 and 210°C steam cracked samples, respectively; SFSE: solid fraction of steam exploded sample; SF: solid fraction; RWCLB: raw Williams Cavendish Lignocellulosic Biomass (dry matter).

presented in Figure 2(b), in which the two highest concentrations of 2F and 5-HMF, expressed as μg ml^{-1} of LF, were 21 and 41 for LFSC210 and 20 and 7 for the liquid fraction of steam exploded (LFSE) sample, respectively.

Our results showed that the formation of 2F (a degradation product of xylose) was directly affected by the severity factor. Similar results were obtained by Vanderghem et al. (2012) in their study of the acid delignification of *Miscanthus x giganteus* for enzymatic hydrolysis. It clearly appears that the formation of 5-HMF was directly affected by temperature, given that its concentrations in the LFSC210 (21 μg ml^{-1}) and LFSE (20 μg ml^{-1}) samples were comparable. The formation of 2F was also affected by the retention time, given that the concentration of 2F in LF of SE pretreated material over a 3 min retention time was less (7 μg ml^{-1}) than the concentration from SC210 treated material over 5.03 min (41 μg ml^{-1}). This is understandable, as an increase in time provides more time for the formation of products, which react with other byproducts through higher order reactions to form new products (Laser et al., 2002). This experiment also reveals that the formation of 2F is more affected by the retention time than the temperature. Furthermore, the furfural contents in LFs assessed in this study were for the most part lower than the inhibition range concentrations (2000–4000 μg ml^{-1}) of *Saccharomyces cerevisiae* and xylose fermenting yeasts (*Candida shehatae* and *Pichia stipitis*), as reported by Weil et al. (2002). 2F and 5-HMF contents, presented in Figure 2(b), are comparable to those of 2F and 5-HMF (250 and 140 μg ml^{-1}, respectively) present in LFs of stage 3 steam pretreated wheat straw at 80°C, 180°C and 190°C at 6, 13 and 3 min, respectively (Kaparaju et al., 2009). Sipos et al. (2010) reported 2F and 5-HMF contents of 510 and 160 μg ml^{-1}, respectively, in LFs of steam pretreated industrial hemp at 210°C with 5 min of retention time after impregnation with a 2% SO_2 solution. The formation of these byproducts during the process implies that the necessary reaction time for the pretreatment of WCLB for ethanol production should be shortened to avoid inhibitor generation. Given that these inhibitors are easily metabolized by anaerobic microbes to produce methane (Barakat et al., 2012), reduction of the reaction time should not be necessary if the goal of the pretreatment is to produce methane through a biomethanation process.

Extractives and lignin contents of RWCLB and SF of treated WCLB

Water and ethanol extractives. As shown in Figure 3(a), the total proportion of water and ethanol extractives, expressed as a percentage of the WCLB DM, was observed with the RWCLB sample (22 %w/w). The proportions of water extractives were the highest in all the studied samples except in the solid fraction of steam exploded (SFSE) sample, in which the proportions of water and ethanol extractives were the same (4 %w/w) (Figure 3(a)).

It was demonstrated by Oliveira et al. (2007) that ash and sugar are the main components of water extractives in dwarf Cavendish banana with proportions ranging between 42.7–68.1 and 14.1–33.3 %w/w, respectively, of water extractive DM. It appears that most of the extractives moved to the LF during pretreatment. This could explain the reductions in extractive proportions in all the pretreated samples compared to the untreated sample. The results also showed that severity factor influenced extractive generation during treatment, as extractive proportions increased with increasing severity factor during SC pretreatment. These proportions ranged from 8 %w/w for SFSC150 to 12 %w/w for SFSC210. The low proportion of extractives from the SFSE could be explained by the severity factor.

Lignin content. As shown in Figure 3(b), the proportions of acid-soluble and insoluble lignin were also affected by the pretreatments. The highest acid-soluble lignin proportions were found in the raw material and in SC pretreated material at 150°C (18 %w/w). The lowest proportions were observed with SFSC210 and SE (6 %w/w).

Table 1. Monosaccharide contents of RWCLB and solid fractions after acid hydrolysis (expressed as %w/w of the dry matter of the fraction).

Neutral sugars	RWCLB		Neutral sugars contents of solid fractions							
			SFSC150[a]		SFSC180[b]		SFSC210[c]		SFSE[d]	
Rhamnose	0.20	±0.00	0.46	±0.02	0.15	±0.03	0.06	±0.02	0.08	±0.01
Arabinose	2.50	±0.00	4.71	±0.37	5.69	±1.49	1.02	±0.15	2.31	±0.19
Xylose	4.05	±0.07	7.46	±0.61	9.40	±2.24	4.53	±0.11	4.76	±0.36
Mannose	0.55	±0.07	1.08	±0.15	1.42	±0.41	1.61	±0.05	0.98	±0.06
Glucose	22.70	±0.71	29.67	±0.00	44.47	±0.00	53.56	±1.33	28.98	±2.12
Galactose	1.10	±0.00	1.33	±0.21	1.66	±0.00	1.03	±0.03	0.62	±0.07
Total	31.10	±0.85	44.70	±1.36	62.79	±4.17	61.82	±1.69	37.71	±2.81

RWCLB: Raw Williams Cavendish Lignocellulosic Biomass.
[a, b, c]Solid fractions of 150, 180 and 210°C steam cracked Williams Cavendish Lignocellulosic Biomass, respectively.
[d]Solid fraction of steam exploded Williams Cavendish Lignocellulosic Biomass.

These results indicated that temperature of the pretreatment procedure greatly influenced lignin solubilization. As described by Bobleter (1994), lignin normally starts to dissolve into water at approximately 180°C under neutral conditions. This assumes that at this temperature (180°C) and above, a portion of acid-soluble lignin undergoes dissolution in LFs. RWCLB was not subjected to pretreatment; therefore, its acid-soluble lignin fraction was unchanged (among the highest). The temperature value (approximately 180°C and up) that allows the dissolution of acid-soluble lignin components could explain why the acid-soluble lignin contents of SC150-treated WCLB and untreated WCLB were the highest (18% of total lignin contents). Furthermore, partial or total dissolution of the acid-soluble lignin portions in the LFs decreases and increases, respectively, the fraction of acid-soluble and acid-insoluble lignin in the distribution of total lignin contents of SFs. This study confirms that the solubilization of the acid soluble lignin fraction during pretreatment depends on the acidification of the reactor contents. Grabber (2005) has demonstrated that the solubility of lignin in acid, neutral or alkaline environments also depends on its unit composition. The solubilization of lignin polymer is linked to its degradation during pretreatment. Barakat et al. (2012) demonstrated the generation of syringaldehyde and vanillin (enzymatic inhibitors) during the partial depolymerization of lignin polymers through syringyl (S) and guaiacyl (G) units, respectively. These authors demonstrated the anaerobic digestion of 2F, 5-HMF, syringaldehyde and vanillin. The measured methane potentials were: 105 ml of CH_4 g^{-1} of vanillin, 430 ml of CH_4 g^{-1} of 2F, 450 ml of CH_4 g^{-1} of 5-HMF and 453 ml of CH_4 g^{-1} of syringaldehyde. Given that LFs contain byproducts that inhibit bioethanol production through a fermentation process, this fraction could be used for biomethane generation.

Monosaccharide contents. To assess the effects of pretreatments on WCLB saccharification, diluted acid hydrolysis was performed as described in the *Monosaccharide composition* section. Neutral sugar (NS) contents were then determined. The NS contents of RWCLB, SFSC150, SFSC180, SFSC210 and SFSE, expressed as %w/w of DM, are presented in Table 1.

In general, glucose presented the highest proportion of NS followed by xylose and arabinose. The highest proportions of glucose were found in SFSC210 (53.56 %w/w) and SFSC180 (44.47 %w/w), while the lowest were found in RWCLB (22.70 %w/w) and SFSE (28.98 %w/w). Xylose represented the highest proportions of pentose with values of 4.05, 7.46, 9.40, 4.53 and 4.76 %w/w, respectively, from RWCLB, SFSC150, SFSC180, SFSC210 and SFSE samples. The NS content of SFSE was the lowest (37.71 %w/w) among the SFs. This could be justified by the low delignification during SE pretreatment, assuming that the lowest retention time (3 min) of the SE process did not enable sufficient deconstruction of WCLB. This result is comparable to those of other sources of lignocellulosic biomass, such as dwarf Cavendish banana and *Miscanthus x giganteus*, studied by Oliveira et al. (2007) and Vanderghem et al. (2012), respectively.

The reported results in Table 1 showed that total sugar contents of RWCLB (31.10 %w/w) were lower than the total sugar contents of the pretreated materials. This could be explained by the fact that acid hydrolysis, as shown in this study, was not sufficient alone to efficiently deconstruct RWCLB, depolymerize cellulose and hemicelluloses and deliver a higher quantity of NSs. This result confirms the fact that temperature, acid concentration and residence time influenced the deconstruction of lignocellulosic biomaterial (Janga et al., 2012). As NS contents of untreated WCLB were the lowest, this study revealed that, prior to diluted acid hydrolysis, SC and SE pretreatments were efficient.

To complete the determination of the monosaccharide contents of pretreated WCLB, NS proportions were determined in LFs. The results are presented in Table 2.

As water deacetylated hemicelluloses during the pretreatment process, the resulting acid catalysed the hydrolytic reactions that cleave glycosidic linkages in hemicelluloses and lignin to generate monomers and oligomers (Laser et al., 2002). As presented in Table 2, the proportion of each NS, expressed as %w/w of LF DM, was less than 1 %w/w, with arabinose having the highest value (0.24, 0.28, 0.92 and 0.90 %w/w for LFSC150, LFSC180, LFSC210 and LFSE, respectively). The highest total NS content was obtained from LFSC210 and LFSE with 1.65 and 1.72 %w/w, respectively.

Table 2. Monosaccharide contents of the liquid fraction after acid hydrolysis (expressed as %w/w of the dry matter of the liquid fraction).

Neutral sugars	Neutral sugar contents of liquid fractions							
	LFSC150[a]		LFSC180[b]		LFSC210[c]		LFSE[d]	
Rhamnose	0.07	±0.01	0.05	±0.00	0.31	±0.13	0.23	±0.09
Arabinose	0.24	±0.02	0.28	±0.01	0.92	±0.01	0.90	±0.04
Xylose	0.04	±0.02	0.03	±0.01	0.14	±0.03	0.21	±0.04
Mannose	0.10	±0.01	0.05	±0.00	0.11	±0.02	0.16	±0.02
Glucose	0.08	±0.00	0.03	±0.01	0.06	±0.01	0.12	±0.03
Galactose	0.02	±0.00	0.02	±0.01	0.11	±0.01	0.10	±0.02
Total	0.55	±0.06	0.46	±0.04	1.65	±0.21	1.72	±0.24

[a, b, c]Liquid fractions of 150, 180, and 210°C steam cracked Williams Cavendish Lignocellulosic Biomass, respectively.
[d]Liquid fraction of steam exploded Williams Cavendish Lignocellulosic Biomass.

Table 3. Total monosaccharide contents of combined liquid and solid fractions (expressed as %w/w of raw Williams Cavendish Lignocellulosic Biomass dry matter).

Neutral sugars	Monosaccharide contents[a] of steam cracked and exploded solid and liquid fractions							
	SLFSC150[b]		SLFSC180[c]		SLFSC210[d]		SLFSE[e]	
Rhamnose	0.39	± 0.02	0.13	±0.03	0.11	±0.04	0.10	±0.02
Arabinose	3.89	±0.31	4.82	±1.25	1.00	±0.12	2.07	±0.16
Xylose	6.12	±0.50	7.90	±1.88	3.65	±0.09	3.98	±0.31
Mannose	0.90	±0.12	1.20	±0.34	1.31	±0.04	0.84	±0.05
Glucose	24.34	±0.00	37.36	±0.00	42.86	±1.07	24.07	±1.76
Galactose	1.09	±0.17	1.40	±0.00	0.85	±0.03	0.53	±0.06
Total	36.73	±1.12	52.82	±3.51	49.78	±1.39	31.59	±2.37

[a]Determined after correction for solid and liquid fraction proportions presented in Figure 2(a) ([b] 0.86 and 0.14; [c] 0.84 and 0.16; [d] 0.80 and 0.20; [e]0.83 and 0.17).

Assuming WCLB acid autohydrolysis, these results indicated that very few monomers generated during the steam process were liberated in LFs. Acid autohydrolysis of delignified holocelluloses at high temperature (210°C) during the steam phase and the shear action of the explosion phase (Ramos et al., 1992) liberate more sugars (monosaccharides and oligosaccharides) in the LFSE than in other LFs. Therefore, the highest NS content (1.72 %w/w of the LF DM) found in the LFSE could be justified by the liberation of more monosaccharides in the LFSE than in other LFs. The higher retention of SC210 (5.02 min) enables the formation of more degradation products, such as 2F and 5-HMF (Figure 2(b)), through the transformation of monosaccharides produced by acid autohydrolysis during the process. This transformation therefore relatively reduces the total monosaccharide content in LFSC210 (1.65 %w/w).

The total NS content in LFSC180 (0.46 %w/w of LF DM) was lower than the content in LFSC150 (0.55 %w/w of LF DM). As presented in Figure 2(b), the sugar degradation product (2F) was higher in LFSC180 (6 μg ml^{-1} of LF) than in LFSC150 (0 μg ml^{-1} of LF). Thus, the low total NS content in LFSC180, as mentioned previously, could be justified by the transformation of a fraction of NS into 2F, consequently decreasing the total NS content in LFSC180 (Table 2).

To assess the full efficiency of the pretreatment, the total NS contents of the LF and SF were expressed as %w/w of untreated WCLB DM. As reported in Table 3, SLFSC180 and SLFSC210 generated the highest proportions of NS contents, respectively, with 52.82 and 49.78 %w/w of RWCLB DM. In terms of polysaccharides, these correspond to 47.54 and 44.80 wt% of RWCLB DM, respectively. SLFSC210 yielded the highest proportion of glucose content (42.86 %w/w of RWCLB DM).

In this study, the temperature and severity factor appear to be important contributors to the efficiency of the diluted acid hydrolysis process, with an optimum range of 180–210°C corresponding to a severity factor of 3.38–4.29. The total NS content of SLFSE was the lowest at 31.59 %w/w. This could be explained by the decreased deconstruction of WCLB during the SE process related to the low retention time of the process (3 min), as mentioned previously.

Bioaccessible glucan and xylan contents. ECD was performed on some SFs and LFs to verify the bioaccessibility and fermentability of SC and SE pretreated WCLB. As presented in Table 4, the content of the most bioaccessible homopolymers (glucan and xylan), representing approximately 35.03 %w/w of RWCLB DM (43.79 %w/w of SF DM), was found in the SFSC210 sample. The lowest, constituting 13.19 %w/w of RWCLB DM, was found in the untreated sample (RWCLB).

Bioaccessible glucan and xylan content in SFSE, representing 23.70 %w/w of RWCLB DM, was less than the bioaccessible glucan and xylan content of the SFSC210 sample reported

Table 4. Bioaccessible sugar contents revealed after enzymatic hydrolysis (expressed as %w/w of fraction dry matter).

Components	Bioaccessible sugar contents of solid and liquid fractions							
	RWCLB		SFSC210		SFSE		LFSE[h]	
Glucan[a]	10.17	±0.47	34.68	±1.00	22.04	±1.69	8.82	±0.46
Xylan[b]	3.02	±0.17	9.11	±0.03	6.52	±0.57	4.96	±0.00
Total	13.19	±0.64	43.79	±1.03	28.56	±2.26	14.11	±0.46
Total[c]	–	–	(35.03	±0.82)[d]	(23.70	±1.88)[e]	(2.40	±0.08)[f]
Glucose	11.29	±0.52	38.49	±1.11	24.46	±1.88	9.79	±0.51
Xylose	3.55	±0.19	10.11	±0.03	7.24	±0.63	5.51	±0.00
Total	14.84	±0.71	48.60	±1.14	31.70	±2.51	15.30	±0.51
Hemicelluloses[g]	3.78	±0.21	11.39	±0.04	8.15	±0.71	6.20	±0.00

[a, b]Deducted from glucose and xylose (taking into consideration H_2O incrementation during the hydrolysis) produced by enzymatic hydrolysis. Glucan and xylan content were assessed according to the following ratios: glucose content/1.11 and xylose content/1.11.
[c]Expressed as %w/w of raw Williams Cavendish Lignocellulosic Biomass (RWCLB) dry matter and determined after correction for the fraction proportions presented in Figure 2(a) ([d] 0.80; [e] 0.83; [f] 0.17).
[g]Estimated bioaccessible hemicelluloses, given that xylose represents approximately 80 %w/w of the sugar content in the hemicellulosic fraction (Chandel et al., 2012).
[h]Corrected for monomer content (glucose and xylose) derived from auto acid autohydrolysis.

earlier. These results confirm that more holocelluloses (cellulose and hemicelluloses) were bioaccessible in SFSC210, assuming that SC210 treatment was more efficient for WCLB deconstruction. In general, the studied physicochemical pretreatments ameliorated holocellulose accession to enzymes, given that monosaccharide (glucose and xylose) content derived from holocellulose enzymatic hydrolysis of untreated biomaterial (RWCLB) was the lowest (14.84 %w/w of RWCLB DM). Without depolymerization of holocelluloses (to facilitate cell wall delignification), delignification (to break down cell wall resistance), decrystallization of the cellulose (to facilitate enzymatic hydrolysis) and reduction of the hemicellulose degree of acetylation, accessibility of enzymes to WCLB components becomes difficult (Kim and Holtzapple, 2006; Kumar et al., 2009; Mosier et al., 2005; Taherzadeh and Karimi, 2008; Zheng et al., 2009). The low bioaccessible contents obtained with SFSE justified insufficient deconstruction of WCLB during the SE process. This demonstrated that SC pretreatment performed in this study for WCLB deconstruction was more efficient than SE. Assessment of the presence of oligomers (oligoglucan and oligoxylan) in the LFSE shows that these molecules are present in this fraction, totalling approximately 2.4 %w/w of RWCLB DM. This confirms the presence of oligomers in LFs. The oligomer content in the LFSE could be explained by incomplete acid autohydrolysis and shear action on cellulose and hemicellulose due to the sudden drop in pressure during the SE process, as described by Ramos et al. (1992).

Conclusions

SC and SE pretreatments showed good potential for delignification, depolymerization, decrystallization and deacetylation of WCLB, therefore enhancing its enzymatic hydrolysis. Steam target temperature and retention time were the most important contributors to the efficiency of the performed physicochemical pretreatments on one hand, and the diluted acid and enzymatic hydrolysis process on the other hand. This study demonstrated that SC210 generated approximately 2.7-fold bioaccessible polysaccharides (glucan and xylan) from WCLB, while SE generated approximately 1.8-fold compared to untreated WCLB. The low amounts of enzymatic inhibitors, such as 2F and 5-HMF, present in the LFs of both steam cracked and exploded WCLB confirms the potential industrial exploitation of these steam processes. The severity factor could be increased for higher deconstruction of WCLB, given the low production of enzymatic inhibitors and their potential digestibility by anaerobic consortia to effectively produce methane. LFs could then be used efficiently as substrates for methane production, while SFs could be exploited for ethanol production in an integrated process. This study also revealed that the optimum temperature and retention time of the performed pretreatments were 210°C and 5.03 min, respectively, corresponding to a 4.29 severity factor. The efficiency of these pretreatments and the effects of the produced enzymatic inhibitors during biochemical processes need to be confirmed during further experiments.

Acknowledgements

The authors would like to thank Prof. Michel Paquot and Dr Jacqueline Destain for their interest and support.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Williams Cavendish BLB was provided by CDC-Del Monte (agro-industrial company based in Cameroon). This work was supported by the Walloon Centre of Industrial Biology (CWBI) of the University of Liege and the department of Industrial Biological Chemistry-ULG-Gembloux Agro-Bio Tech.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

Barakat A, Monlau F, Steyer J-Ph, et al. (2012) Effect of lignin-derived and furan compounds found in lignocellulosic hydrolysates on biomethane production. *Bioresource Technology* 104: 90–99.

Blakeney AB, Harris PJ, Henry RJ, et al. (1983) A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research* 113: 291–299.

Bobleter O (1994) Hydrothermal degradation of polymers derived from plants. *Progress in Polymer Science* 19: 797–841.

Brownell HH and Saddler JN (1987) Steam pretreatment of lignocellulosic material for enhanced enzymatic hydrolysis. *Biotechnology and Bioengineering* 29: 228–235.

Carreel F, Gonzalez de, Leon D, Lagoda PJL, et al. (2002) Ascertaining maternal and paternal lineage within musa by chloroplast and mitochondrial dna rflp analyses. *Genome* 45: 679–692.

Carvalheiro F, Duarte LC and Grio FM (2008) Hemicelluloses biorefineries: a review on biomass pretreatments. *Journal of Scientific and Industrial Research* 67: 849–864.

Chandel AK, Antunes FAF, De Arruda PV, et al. (2012) Dilute acid hydrolysis of agro-residues for the depolymerization of hemicelluloses: State-of-the-art. In: Da Silva SS and Chandel AK (eds) *d-xylitol*. Berlin: Springer-Verlag Berlin Heidelberg, pp.39–61.

Clark TA, Mackie KL, Dare PH, et al. (1989) Steam explosion of the softwood pinus radiata with sulphur dioxide addition. II. Process characterisation. *Journal of Wood Chemistry and Technology* 9: 135–166.

Demirbas A (2011) Competitive liquid biofuels from biomass. *Applied Energy* 88: 17–28.

Didderen I, Destain J and Thonart P (2008) Le bioéthanol de seconde génération: La production d'éthanol à partir de biomasse lignocellulosique. *Les Presses agronomiques de Gembloux*, Belgique.

Ehrman T (1996) Determination of acid-soluble lignin in biomass. Golden, CO: Laboratory analytical procedure 4.

FAO(2013) FAOSTAT, statistics data base. Agriculture. FAO, Rome.

Fischer G and Heilig GK (1997) Population momentum and the demand on land and water resources. *Philosophical Transactions of the Royal Society of London. Series B. Biological Sciences* 352: 869–889.

Global Musa Genomics Consortium (2001) A strategy for the Global Musa Genomics Consortium. In: *the international network for the improvement of banana and plantain, Montpellier, France. INIBAP ISBN*, 2–910810, report of a meeting held in Arlington, USA, 17–20 July 2001.

Grabber JH (2005) How do lignin composition, structure, and cross-linking affect degradability? A review of cell wall model studies. *Crop Science* 45: 820–831.

Han G, Deng J, Zhang S, et al. (2010) Effect of steam explosion treatment on characteristics of wheat straw. *Industrial Crops and Products* 31: 28–33.

Happi ET, Wathelet B and Paquot M (2008) Changements texturaux et biochimiques des fruits du bananier au cours de la maturation. Leur influence sur la preservation de la qualité du fruit et la maitrise de la maturation. *Biotechnology Agronomy Society and Environment* 12: 89–98.

Hendriks ATWM and Zeeman G (2009) Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology* 100: 10–18.

Hiligsmann S, Beckers L, Masset J, et al. (2011) La production de biohydrogène à partir de substrats carbohydratés: état de l'art. *Récents progrès en génie des procédés*. Available at: http://hdl.handle.net/2268/112556. (accessed 04 November 2014).

Honfo GF, Tenkouano A and Coulibaly O (2011) Banana and plantain-based foods consumption by children and mothers in Cameroon and southern Nigeria: a comparative study. *Afrcan Journal of Food Science* 5: 287–291.

Ibrahim MM, Agblevor FA and El-Zawawy WK (2010) Isolation and characterization of cellulose and lignin from steam-exploded lignocellulosic biomass. *Bioresources* 5: 397–418.

INIBAP(1999) Networking banana and plantain: Inibap annual report 1998. Technical report, International Network for the Improvement of Banana and Plantain, Monpellier, France.

Jacquet N (2012) Impact de la steam explosion et de l'homogeneisation sur les proprietes physicochimiques et l'hydrolyse enzymatique de la cellulose. Available at: http://orbi.ulg.ac.be/handle/2268/134996 (accessed 20 December 2014).

Jacquet N, Quiévy N, Vanderghem C, et al. (2011) Influence of steam explosion on the thermal stability of cellulose fibres. *Polymer Degradation and Stability* 96: 1582–1588.

Jacquet N, Vanderghem C, Blecker C, et al. (2010) La steam explosion: Application en tant que prétraitement de la matière lignocellulosique. *Revue de Biotechnologie, Agronomie Société et Environnement* 14(spécial 2): 561–566.

Jacquet N, Vanderghem C, Danthine S, et al. (2012) Influence of steam explosion on physicochemical properties and hydrolysis rate of pure cellulose fibers. *Bioresource Technology* 121: 221–227.

Janga KK, Hagg M-B and Moe ST (2012) Influence of acid concentration, temperature, and time on decrystallization in two-stage concentrated sulphuric acid hydrolysis of pinewood and aspenwood: a statistical approach. *Bioresources* 7: 391–411.

Kamdem I, Hiligsmann S, Vanderghem C, et al. (2013) Comparative biochemical analysis during the anaerobic digestion of lignocellulosic biomass from six morphological parts of williams cavendish banana (triploid musa AAA group) plants. *World Journal of Microbiology and Biotechnology* 29: 2259–2270.

Kamdem I, Tomekpe K and Thonart Ph (2011). Production potentielle de bioéthanol, de biomthane et de pellets partir des déchets de biomasse lignocellulosique du bananier (musa spp.) au Cameroun. *Biotechnology Agronomy Society and Environment* 15: 461–473.

Kaparaju P, Serrano M, Thomsen AB, et al. (2009) Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept. *Bioresource Technology* 100: 2562–2568.

Kim S and Holtzapple MT (2006) Effect of structural features on enzyme digestibility of corn stover. *Bioresource Technology* 97: 583–591.

Klinke HB, Thomsen AB and Ahring BK (2004) Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Applied Microbiology and Biotechnology* 66: 10–26.

Kumar P, Barrett DM, Delwiche MJ, et al. (2009) Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial and Engineering Chemistry Research* 48: 3713–3729.

Laser M, Schulman D, Allen SG, et al. (2002) A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol. *Bioresource Technology* 81: 33–44.

Lassois L, Busogoro JP and Jijakli H (2009) La banane: De son origine à sa commercialisation. *Biotechnology Agronomy Society and Environment* 13: 575–586.

Lassoudière A (2007). *Le Bananier et sa Culture*. Versailles: Quae.

Lassoudière A (2012) *Le Bananier: Un Siècle D'innovations Techniques*. Versailles: Quae, Quae.

Laurent P, Roiz J, Wertz JL, et al. (2011) Le bioraffinage, une alternative prometteuse à la pétrochimie. *Biotechnology Agronomy Society and Environment* 15: 597–610.

Lechien V, Rodriguez C, Ongena M, et al. (2006) Physicochemical and biochemical characterization of non-biodegradable cellulose in Miocene gymnosperm wood from the entre-sambre-et-meuse, southern Belgium. *Organic Geochemistry* 37: 1465–1476.

Li H and Chen H (2008) Detoxification of steam-exploded corn straw produced by an industrial-scale reactor. *Process Biochemistry* 43: 1447–1451.

Martin-Sampedro R, Martin JA, Eugenio ME, et al. (2011) Steam explosion treatment of Eucalyptus globulus wood: influence of operational conditions on chemical and structural modifications. *Bioresources* 6: 4922–4935.

Mok SLW and Antal MJ Jr (1992) Uncatalyzed solvolysis of whole biomass hemicelluloses by hot compressed liquid water. *Industrial and Engineering Chemistry Research* 31: 1157–1161.

Mosier N, Wyman C, Dale B, et al. (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology* 96: 673–686.

Ogier JC, Ballerini D, Leygue JP, et al. (1999) Production d'éthanol partir de biomasse lignocellulosique. *OilGas Science and Technology* 54: 67–94.

Oliveira L, Cordeiro N, Evtuguin DV, et al. (2007) Chemical composition of different morphological parts from dwarf cavendish banana plant and their potential as non-wood renewable source of natural products. *Industrial Crops and Products* 26: 163–172.

Overend RP, Chornet E and Gascoigne JA (1987) Fractionation of lignocellulosics by steam-aqueous pretreatments. *PhilosTransactions of the Royal Society A* 321: 523–536.

Pienkos PT and Zhang M (2009) Role of pretreatment and conditioning processes on toxicity of lignocellulosic biomass hydrolysates. *Cellulose* 16: 743–762.

Ramos LP, Breuil C, Kushner DJ, et al. (1992) Steam pretreatment conditions for effective enzymatic hydrolysis and recovery yields of eucalyptus viminalis wood chips. *International Journal of Biology, Chemistry, Physics, and Technology of Wood* 46:149–154.

Rodriguez C (2005) *Activité biologique dans les centres d'enfoiussement technique de déchets ménagers: biodisponibilité de la cellulose et modélisation*,(Doctoral dissertation, PhD Thesis, Université de Liege, Centre Wallon de Biologie Industrielle.

Rodriguez C, Hiligsmann S, Ongena M, et al. (2005) Development of an enzymatic assay for the determination of cellulose bioavailability in municipal solid waste. *Biodegradation* 16: 415–422.

Shepherd K (1999) Cytogenetics of the genus Musa. International Network for the Improvement of Banana and Plantain, Monpellier, France, p.160.

Simmonds NW and Shepherd K (1955) The taxonomy and origins of the cultivated bananas. *Journal of the Linnean Society London. Botany* 55: 302–312.

Sipos B, Kreuger E, Svensson SE, et al. (2010) Steam pretreatment of dry and ensiled industrial hemp for ethanol production. *Biomass and Bioenergy* 34: 1721–1731.

Sluiter A, Hames B, Ruiz R, et al. (2008) Determination of structural carbohydrates and lignin in biomass. In: *laboratory analytical procedure*, National Renewable Energy Laboratory, Golden, CO, USA.

Sluiter A, Ruiz R, Scarlata C, et al. (2005) Templeton D. Determination of extractives in biomass. In: *laboratory analytical procedure*, National Renewable Energy Laboratory, Golden, CO, USA.

Taherzadeh MJ and Karimi K (2008) Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. *International Journal of Molecular Sciences* 9: 1621–1651.

Vanderghem C, Brostaux Y, Jacquet N, et al. (2012) Optimization of formic/acetic acid delignification of miscanthus × giganteus for enzymatic hydrolysis using response surface methodology. *Industrial Crops and Products* 35: 280–286.

Van Walsum GP, Allen SG, Spencer MJ, et al. (1996) Conversion of lignocellulosics pretreated with liquid hot water to ethanol. *Applied Biochemistry and Biotechnology* 57: 157–170.

Weil JR, Dien B, Bothast R, et al. (2002) Removal of fermentation inhibitors formed during pretreatment of biomass by polymeric adsorbents. *Industrial and Engineering Chemistry Research* 41: 6132–6138.

Wertz JL (2012) *Prétraitement de la biomasse lignocellulosique*. 9ième rencontre de la biomasse Rapport de synthèse, Document ValBiom-Gembloux AgroBioTech.

Zheng Y, Pan Z and Zhang R (2009) Overview of biomass pretreatment for cellulosic ethanol production. *International Journal of Agricultural and Biological Engineering* 2: 51–68.