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A new lignocellulosic biomass deconstruction process combining thermo-mechano chemical action and bio-catalytic enzymatic hydrolysis in a twin-screw extruder

Virginie Vandenbossche[a,b,*], Julien Brault[a,b], Gérard Vilarem[a,b], Oscar Hernández-Meléndez[c], Eduardo Vivaldo-Lima[c], Martín Hernández-Luna[c], Eduardo Barzana[c], Aleta Duque[d], Paloma Manzanares[d], Mercedes Ballesteros[d], Julio Mata[e], Erick Castellón[e], Luc Rigal[a,b]

[a] Université de Toulouse, INP, Laboratoire de Chimie Agro-industrielle, ENSIACET, 4 Allée Emile Monso, BP 44362, 31030 Toulouse Cedex 4, France
[b] INRA, Laboratoire de Chimie Agro-industrielle, 31030 Toulouse Cedex 4, France
[c] Facultad de Quimica, Universidad National Autónoma de México, 04510 México, DF, Mexico
[d] Biofuels Unit, Energy Department – CIEMAT, Avda. Complutense, 22 28040 Madrid, Spain
[e] Labotorio de Quimica Biorganica, Escuela de Quimica, Facultad de Ciencias, Universidad de Costa Rica, San José, Costa Rica

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ABSTRACT

A new process for the deconstruction of lignocellulosic biomass was evaluated. It combines thermo-mechano-chemical action and the bio-catalytic action of enzymatic hydrolysis in a twin-screw extruder. The process proceeds in three phases all conducted in a twin-screw extruder: an alkaline pretreatment, a neutralization phase and an enzyme impregnation phase during which hemicelluloses and cellulose saccharification begins. Four model feedstocks with high cellulose content have been selected for the study: sweet corn residue (SC), a co-product of industrial corn grain canning; blue agave bagasse (BAB) from the manufacture of tequila; oil palm empty fruit bunch (OPEFB), a residue from palm oil manufacture, and barley straw (BS). They are all agricultural or agro-industrial processing by-products, although their morphological origin, chemical composition and physical structure differ. Their differences in behavior are studied throughout the process, and their cell wall constituents, hemicelluloses, cellulose and lignin, quantified. The enzymatic hydrolysis of the four feedstocks ranged from 8 to 26% of dry matter and reached 26 to 68% of dry matter after alkaline pretreatment depending on the particular biomass. The process allows preparation of high dry matter content (>20%) cellulosic material for saccharification. The continuous treatment allows extraction of a large part of the hemicelluloses (up to 64% for SC), configures cellulose for better accessibility, and initiates cellulose depolymerization by the enzyme cocktail during bioextrusion. This new process is advantageous because it minimizes energy consumption by operating at a low temperature, minimizes water consumption by working at a low liquid/solid ratio and is fast and adaptable to different biomasses.

1. Introduction

With increasing energy demands, increasing CO_2 emissions and decreasing fossil energy, it is necessary to resort to other energy sources. Residual lignocellulosic materials from agriculture and forestry by-products represent an important source of energy. They are clean, cheap, available in large quantities, are independent of geographical location, plus they are carbon neutral and renewable. Lignocellulosic biomass transformation processes producing second generation bioethanol are studied worldwide, although the substrate is recalcitrant to enzyme accessibility (Himmel et al., 2007) and requires the use of a pretreatment. The key limiting factors of the different processes are: the pretreatment and the enzymatic hydrolysis efficiency, cost, and the C5–C6 co-fermentation.

* Corresponding author at: Université de Toulouse, INP, Laboratoire de Chimie Agro-industrielle, ENSIACET, 4 Allée Emile Monso, BP 44362, 31030 Toulouse Cedex 4, France. Tel.: +33 5 34 32 35 49; fax: +33 5 34 32 35 99.
E-mail address: Virginie.Vandenbossche@ensiacet.fr (V. Vandenbossche).

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1.1. Pretreatment for lignocellulosic ethanol

The pretreatment has a very important role. Simple and as cheap as possible, it must reduce particle size and improve enzyme accessibility, as well as limit formation of toxic compounds that inhibit fermentation. Merely reducing size mechanically is an energy-intensive process and insufficient to obtain acceptable cellulose digestibility (Himmel et al., 2007). Pore size is a major limiting factor for the enzymatic hydrolysis of cellulose (Chandra et al., 2007), and an increase in porosity can be obtained by removing the hemicelluloses. This increases substrate accessibility to cellulases and in turn increases the likelihood of hydrolyzing the cellulose. Other factors such as cellulose crystallinity (Chang and Holtzapple, 2000) or lignin content (Mansfield et al., 1999) are also important to obtain good digestibility.

Ogier et al., 1999 reviewed feasible lignocellulosic biomass pretreatment methods for ethanol production, and many of these have already been studied, such as: hot water (Mosier et al., 2005; Laser et al., 2002; Pérez et al., 2008), steam explosion (Oliva et al., 2003; Cara et al., 2006; Varga et al., 2004; Ballesteros et al., 2006), alkaline pretreatment (Carvalheiro et al., 2008; Kumar et al., 2009; McIntosh and Vancov, 2011; Taherzadeh and Karimi, 2008), alkaline pretreatment with an oxidant (Carvalheiro et al., 2008), pretreatment in organic solvents such as glycerol or alkaline-glycerol (Adeeb, 2004), dilute acid pretreatment (Saha et al., 2005), ammonia fiber explosion (Galbe and Zacchi, 2007), oxidation pretreatment, microwave pretreatment (Keshwani, 2009), ultrasound pretreatment (Yachmenev et al., 2009), and supercritical CO_2 pretreatment (Kim and Hong, 2001). Those pretreatments most cited are often penalized by implementation constraints, technology or reagent costs, or inhibitor production for enzymatic hydrolysis or ethanolic fermentation. Despite the inevitable reagent costs of any chemical treatment, alkaline pretreatments in particular present very few drawbacks.

1.2. Twin-screw extrusion

Among the processes used to carry out pretreatment with a minimum number of steps, extrusion has many advantages. It produces a high shear, rapid heat transfer, and effective and rapid mixing, in a continuous operation, with good modulation of treatment steps.

Co-penetrating and co-rotating twin-screw extruders are most common (Dziezak, 1989), and a very wide choice of screw elements is available. The screw profile (or screw configuration) defines the arrangement of the different screw elements and their characteristics (pitch, stagger angle, and length) in different positions and spaced differently. It is the main factor influencing performance (product transformation, residence time distribution, and mechanical energy input) during extrusion processing (Gogoi et al., 1996; Gautam and Choudhury, 1999a, 1999b).

The performance of extrusion and the influence of the operating parameters have been studied on biomass from poplar (N'Diaye and Rigal, 2000), *Miscanthus* sp. (De Vrije et al., 2002), sugar beet pulp (Rouilly et al., 2006), sunflower (Evon et al., 2007), soybean hulls (Yoo et al., 2011), rice straw (Chen et al., 2011), and wheat straw and bran (Marechal et al., 2004; Zeitoun et al., 2010; Jacquemin et al., 2012).

More particularly, extrusion can be used to pretreat different biomasses for the production of sugars, and several authors have reported this type of application over the last few years.

Lamsal et al., 2010 showed that extrusion led to higher reducing sugar yields compared to grinding, and they studied the process on wheat bran in two steps: impregnation with water followed by mechanical treatment (grinding or extrusion). Lee et al., 2010 used extrusion after hot-compressed water treatment and obtained a greatly improved monosaccharide production yield with Douglas fir and Eucalyptus. They also used ethylene glycol, glycerol and DMSO to open up the cell wall structure and improve enzymatic accessibility (Lee et al., 2009).

The combination of extrusion with chemical treatment can also improve results. A pretreatment in a dilute acid medium at low temperature can reach the wall structure by hydrolysis of certain components, especially hemicelluloses. This combination of extrusion and dilute acid pretreatment has been tested on rice straw (Chen et al., 2011).

Aqueous alkaline treatments can reach the fiber structure by solvation of certain components, accompanied by the solubilization and the extraction of hemicelluloses and lignins, to a greater or lesser degree depending on the operating conditions (alkali concentration, temperature, contact time) (Mosier et al., 2005; Balat and Balat, 2008; Zhao et al., 2008). Alkaline pretreatment, which also decreases cellulose crystallinity while increasing its swelling, is one of the most widely used methods for enhancing enzymatic digestibility of the lignocellulosic biomass. A combination of both extrusion and alkaline pretreatment has been explored more recently (Zhang et al., 2012; Karunanithy and Muthukumarappan, 2011; Duque et al., 2013). Karunanithy and Muthukumarappan, 2013 provide an overview of this type of thermo-mechanical pretreatment, including the mechanism influencing extruder and feedstock parameters, plus evaluation of pretreatment efficiency. In all the studies described, saccharification is always carried out in a second phase, in a stirred reactor, after neutralization of the medium and pH adjustment outside the extruder.

A continuous process combining alkaline thermo-mechano-chemical pretreatment, followed by injection of enzymes into the twin-screw extruder, called "bioextrusion", has been developed in this study.

2. Experimental

2.1. Materials

2.1.1. Feedstocks

Sweet corn (*Zea mays* L. *Saccharata*) co-products (SC) came from industrial corn grain canneries and were provided, dehydrated, by SARL Soupro+ (Castelmoron sur Lot, France). They were milled using a hammer mill fitted with a 6 mm grid.

Blue agave (*Agave tequilana*) bagasse (BAB) is the fiber residue from the manufacture of Tequila. It was air dried, and kindly provided by the PATRON Spirits Company in Mexico (Atotonilco, State of Jalisco). It was milled using a hammer mill fitted with a 2 mm grid.

Oil palm (*Elaeis guineensis Jasq.*) empty fruit bunch (OPEFB) is the bunch residue after separation of the fruits for the manufacture of palm oil. It was air dried before being sent from Costa Rica (Palma Tica grupo NUMAR), and was milled using a hammer mill fitted with a 2 mm grid.

Barley (*Hordeum vulgare* L.) straw (BS) came from Spain and was provided by the Centre for the Development of Renewable Energy Sources (CEDER – Soria, Spain). It was milled using a hammer mill fitted with a 5 mm grid.

2.1.2. Enzyme cocktails

Enzymatic saccharification was conducted using three hydrolytic enzyme cocktails based on preliminary studies to select the best cocktail for each feedstock. These three cocktails, *A*, *N*1 and *N*2, are mixtures of cellulase and hemicellulases. *A* is used for SC and OPEFB, *N*1 and *N*2 are used respectively for BS and for BAB.

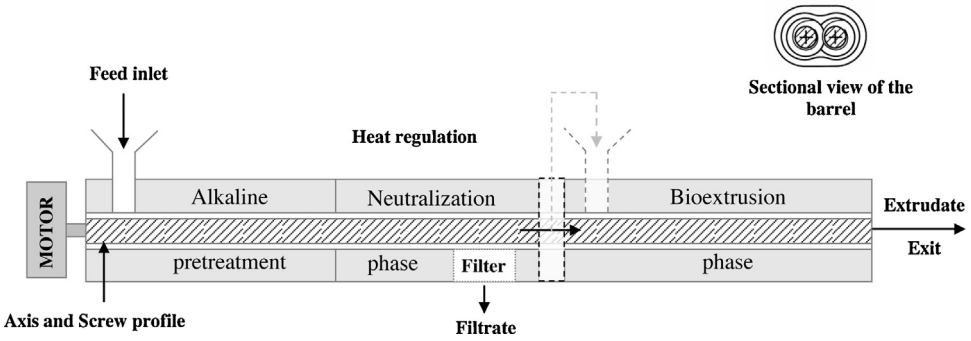


Fig. 1. Schematic representation of the sequence of steps carried out in two successive twin-screw extruders or in a single twin-screw extruder.

2.2. Twin-screw extruder

Thermo-mechano-chemical alkaline pretreatment plus a neutralization phase were conducted using 3 co-penetrating and co-rotating twin-screw extruders: a Clextral BC 45 for SC and OPEFB in France, and two Clextral EV 25, one for BS in Spain and the other for BAB in Mexico. For the bioextrusion phase, a Clextral BC 21 was used for SC and OPEFB in France, and two Clextral EV25, one for BS in Spain and the other for BAB in Mexico. BC 45 and BC 21 had barrels made up of seven modules, EV 25, barrels of six modules for BS and ten modules for BAB, and these modules were 200 mm long for BC 45 and 100 mm for BC21 and EV 25. The extruders were equipped with different segmental screw elements. Modules were thermo-regulated by thermal induction for BC45 and heater band for BC21 and EV25, and cooled by water circulation. A filter section was used to enable the filtrate to be collected, and this consisted of six hemispherical dishes with conical holes (1 mm entry, 2 mm exit). A schematic representation of the sequence of steps for the processes carried out in two successive twin screw extruders (SC, OPEFB, BS) or using a single machine (BAB) is shown in Fig. 1. Screw configuration for the combined processes of pretreatment and bioextrusion of model feedstocks is shown in Fig. 2.

2.2.1. Alkaline thermo-mechano-chemical pretreatment and neutralization phase

The operational parameters for alkaline extrusion were adapted for each model feedstock. The pretreatment alkali is sodium hydroxide (NaOH), and the neutralization step uses phosphoric acid (H_3PO_4). Operating conditions for each feedstock are shown in Table 1.

Feedstocks were fed into the extruder's first module using a volumetric screw feeder, and the alkaline solution was injected using a piston pump. A first zone of mechanical pressure, consisting of a succession of bilobal paddles, ensures grinding and good mixing of the material with the alkaline solution. For OPEFB, an additional mechanical pressure step (bilobe paddles) before alkali injection, is necessary to avoid liquid return back along the barrel to the in-feed zone.

An acid solution was injected using a piston pump to neutralize the medium and reduce the viscosity of the matter to ensure good filtration. A second zone of mechanical pressure ensures good mixing with the medium, and fast neutralization. The filtration zone, situated in modules 5 or 6, depending on the feedstock, was ensured by a third zone of mechanical pressure using reverse pitch screws.

Table 1
Operating conditions.

Operating conditions	SC	BS	BAB	OPEFB
Alkaline pretreatment				
S_S (rpm)	110	150	85	200
T (°C)	100	68	75	98
NaOH/DM (%)	8.5	7.5	8.6	12.6
L/S	8	12	3.2	8.2
Bioextrusion				
S_S (rpm)	250	150	85	200
T (°C)	50	50	50	40
Enzyme cocktails	A	N_1	N_2	A
Enzyme/DM (%)	2.5	2.5	2	11.0
L/S	3.1	4	4.35	3.1

DM: dry matter, SC: sweet corn, BS: barley straw, BAB: Blue agave bagasse, OPEFB: oil palm empty fruit bunch, L/S: liquid/solid ratio.

2.2.2. Bioextrusion

Bioextrusion consists of introducing an enzyme cocktail, selected as a function of the feedstock, into the extruder, and using a succession of compression and expansion phases to facilitate the penetration of these enzymes into the matter. Operating conditions are shown for each feedstock in Table 1. The bioextrusion is carried out in continuous mode in the same extruder for BAB, and in consecutive runs in a second extruder for SC, OPEFB and BS.

2.3. Analytical methods

2.3.1. Dry matter and cell wall components

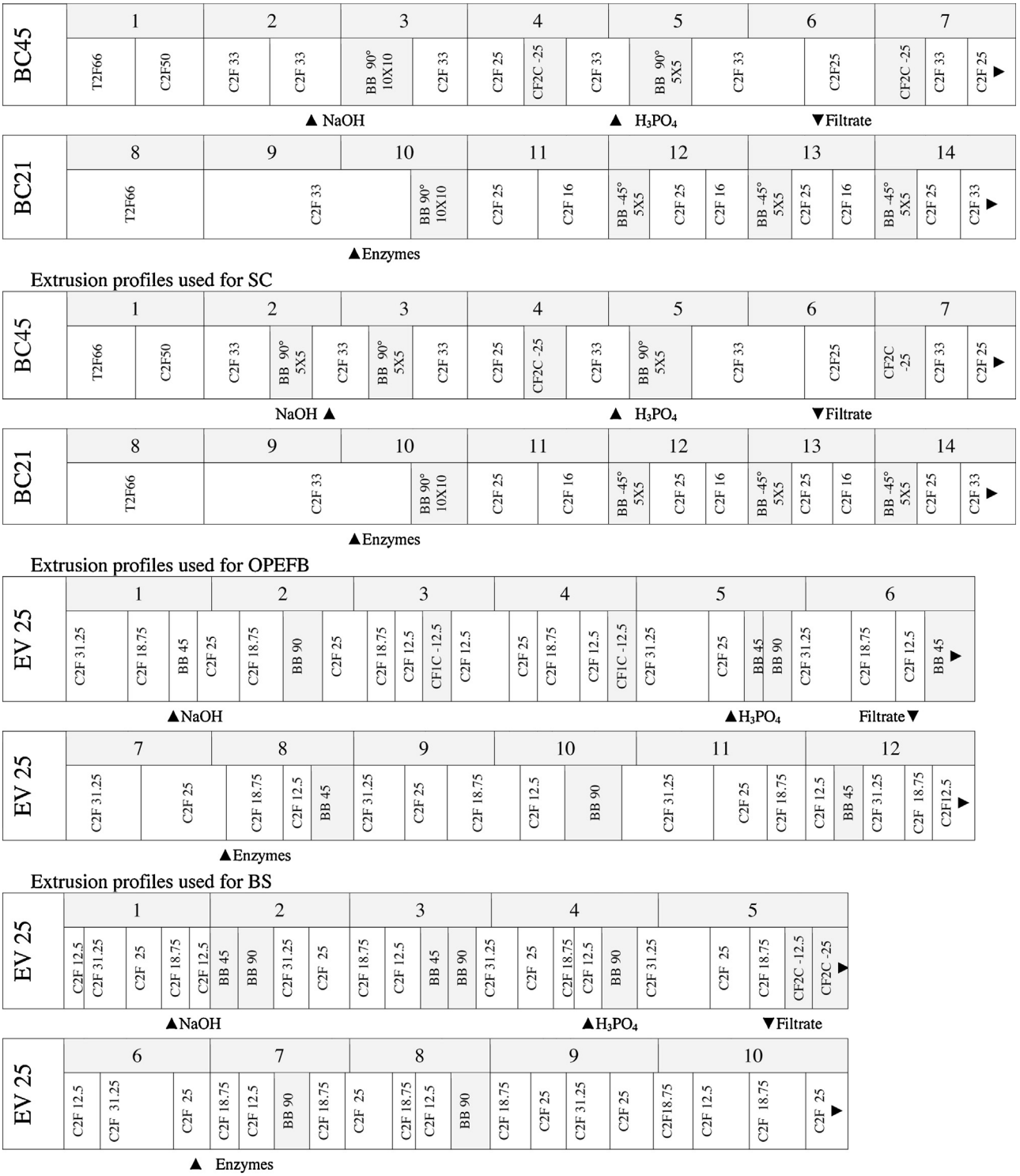
Moisture content was determined according to French standard NF V 03-903. Mineral contents were determined according to French standard NF V 03-322. The ADF-NDF method of Van Soest and Wine, 1967, 1968 was used to estimate the three cell wall constituents (cellulose, hemicelluloses, and lignins) contained in the solids. NDF solubility was also extrapolated.

An estimation of the water-soluble components contained in solids, was made by measuring the mass loss of the test sample after 1 h in boiling water. All determinations were carried out in duplicate.

The lipid content was determined according to the French standard NF V 03-908. The protein content was determined using French standard NF V 18-100.

Measurements of X-ray diffraction were performed by X-ray powder Bruker D8 Advance diffractometer using **Cu-Ka1** and **Cu-Ka2** in a 2θ range from 5° to 60° with a increment of 0.019° per step and a step equivalent speed of 167 s. The crystallinity index was calculated according to Segal's method (Segal et al., 1959). The percentage of type II cellulose was found after deconvolution of the "crystalline" peak centered at $2\theta = 22°$ (Maple® software).

Feedstock hydrolysability has been determined by enzymatic hydrolysis in 50 mM citrate phosphate buffer (pH 4.6) in the presence of Celluclast 1.5 L (16%/DM substrate) and Cellobiase N 188 (4%/DM substrate) at 50 °C during 48 h. It has been calculated as the percentage of sugars released by enzymatic hydrolysis relative



SC: sweet corn, BS: Barley straw, BAB: Blue agave bagasse, OPEFB: Oil palm empty fruit bunch, T2F: trapezoidal double-thread screw, C2F: conveying double-thread screw, BB: Bilobe paddle screw, CF2C: reverse pitch double-thread screw. The numbers following the type of the screw indicate the pitch of T2F, C2F, and CF2C screws or the angle between two successive elements in the case of BB screws

Fig. 2. Screw configuration for the combined process of pretreatment and bioextrusion of model feedstock.

to dry matter. Released sugars were assessed by determination of reducing sugars using the DNS method (Miller, 1959).

After bioextrusion, enzymatic hydrolysis (EH) of the bioextrudate was extended for 48 h at a 2.5% extrudate dilution consistency. Glucose and xylose released were measured for BS, by HPLC (Waters 2695 liquid chromatograph with refractive index detector and AMINEX HPX-87P column) and for SC, OPEFB and BAB, by HPLIC (DIONEX ICS-3000 coupled with pulsed amperometric detection

Table 2
Composition of raw biomass selected as model feedstock.

Raw biomass (%/dry matter)*	SC	BS	BAB	OPEFB
Ash*	4	7	3.9	3.4
Cellulose*	38.6	37.6	38.9	44.2
Hemicelluloses*	36.1	32.4	16.9	24.5
Lignin*	3.8	8.3	18.4	19.2
C (C + H + L)	49.2%	48.0%	52.4%	50.3%
Hot water soluble*	14.4	15.13	11.9	9.95
NDF reagent soluble*	21.4	21.6	25.8	12.2
Organic NDF reagent soluble*	19.3	16.9	21.8	10.9
Proteins*	6	3.6	3	3
Lipids*	2	1.9	0.8	6

SC: sweet corn, BS: Barley straw, BAB: Blue agave bagasse, OPEFB: oil palm empty fruit bunch.

Table 3
Hydrolysability of raw biomass selected as model feedstock (as percentage of reducing sugars relative to dry matter).

Raw biomass	SC	BS	BAB	OPEFB
Hydrolysability (% of DM)	26	17	15	8

DM: dry matter, SC: sweet corn, BS: Barley straw, BAB: Blue agave bagasse, OPEFB: oil palm empty fruit bunch.

(PAD) and fitted with a Carbopac PA1 column). To assess EH yield, glucose released after 48 h EH was referred to the amount of dry bioextrudate used in the incubation run and expressed in g glucose/100 g dry bioextrudate.

3. Results and discussion

3.1. Feedstocks

Among potential industrial feedstocks, four have been chosen as models of lignocellulosic structure to study the innovative transformation process presented. They are available and accessible as by-products from agricultural or agro industrial transformation processes. They have been chosen for their wide variation in properties: morphological origin (fibers of stalks, leaf, cob and bunch), physical structure (Fig. 3), chemical composition (Table 2), and physical cellulose structure. BAB and OPEFB have very fibrous structures (Table 4) and similar appearance after grinding, with very hard compact fibers and fine dust particles (in larger amounts in the case of BAB). SC has a heterogeneous fibrous and granular structure with hard particles of cob. BS has a typical straw structure made of homogeneous flat fibers. Compared to BAB, SC contained more hemicelluloses and less lignin, while BS and OPEFB in turn contained more lignin than SC. OPEFB contained residual oil (6%) which came from the palm oil extraction process. The crystallinity index measurements indicate that OPEFB has the highest value (50%), while BAB has a surprisingly low value of 27% (Table 4), which is probably due to the presence of fine particles from the pulp of the agave, very different from the structure of the fiber. Measuring the crystallinity of the fiber alone would probably give a higher index value.

The enzymatic hydrolysability of the raw feedstocks is low (Table 3), although there are differences among the four selected. SC at 26% is much more hydrolysable than OPEFB at 8%, while BS and BAB have intermediate values of 17% and 15% respectively. This low hydrolysability is related to the enzymatic accessibility, which differs for each feedstock, depending on their composition and physiological characteristics.

The study of these different models will allow the process to be adapted for other comparable feedstocks.

Table 4
Cellulose crystallinity characterization of raw and pretreated biomass selected as model feedstock.

	SC	BS	BAB	OPEFB
Raw biomass				
Crystallinity (%)	41 ± 3	44 ± 8	27 ± 7	50 ± 8
Cellulose type II (%)	8	18	<1	4
Pretreated biomass				
Crystallinity (%)	47 ± 6	46 ± 2	52 ± 1	51 ± 7
Cellulose type II (%)	12	28	7	8

SC: sweet corn, BS: Barley straw, BAB: Blue agave bagasse, OPEFB: oil palm empty fruit bunch.

3.2. Process

The study of the process has been carried out in parallel in three different laboratories from different countries, using a single or two consecutive extruders, and covers three different operations: an alkaline pretreatment, a neutralization phase and an enzyme impregnation phase during which hemicelluloses and cellulose saccharification begins. All of these are carried out in the twin-screw extruder.

3.2.1. Alkaline pretreatment and neutralization phase

The first step of the process is the destructuration of cellulosic material under alkaline conditions.

The alkaline extraction conditions of hemicelluloses and lignin in twin-screw extruders have been widely studied, for instance on sorghum and poplar wood fibers (N'Diaye and Rigal, 2009) or on wheat straw (Magro, 1995; Maréchal, 2001; Zeitoun et al., 2010; Vandenbossche et al., 2014), and this previous work has allowed us to define the configuration and screw profiles used in this study. The aim of this first step is to open up the complex structure of the biomasses, and facilitate access of the hydrolytic enzymes to polysaccharides by increasing surface area (Karunanithy and Muthukumarappan, 2011) and porosity (Zhang et al., 2012).

The thermomechanical effect of the flow restricting elements of the screw profile ensures physical disintegration of the material by separating the fiber bundles. The presence of sodium hydroxide ensures additional chemical destructuration by solubilization of organic matter, especially for hemicelluloses and lignin, depending on the amount used.

The neutralization phase is necessary to obtain a pH compatible with effective enzyme activity during bioextrusion. Phosphoric acid is used in this step because it is triprotic and so limits the mass of acid needed. This neutralization step also decreases the viscosity of the matter by a dilution effect which facilitates filtration and plays a part in developing friction, shear and impacts residence time.

The extraction of organic compounds, solubilized by caustic soda, is possible after neutralization by using a filtration zone where the mixture is pressed and the extracts forced through a filter by the reverse pitch screws. The effectiveness of the filtration step is characterized by measuring the dry matter content of the extrudate obtained, with a high value indicating more efficient pressing.

The efficiency of the filter also depends on the nature of the biomass treated. Depending on its physical structure, the dynamic plug formed in the filtration zone will be more or less hard and thus the filtration more or less effective. For materials such as SC and BS with less, hard structural fibers, pressing is only partial, filtration efficiency is poor and the dry matter after filtration is only 38 and 37% respectively. This results in the hot water soluble fraction remaining high (15.9% for SC and 17.3% for BS). Conversely, in the case of BAB and OPEFB, the raw materials are physically similar, with structured, and harder fibers (Fig. 3), and the dynamic plug is very strong which produces powerful pressing during the filtration step and good physical defibration (Fig. 3). The extrudate dry

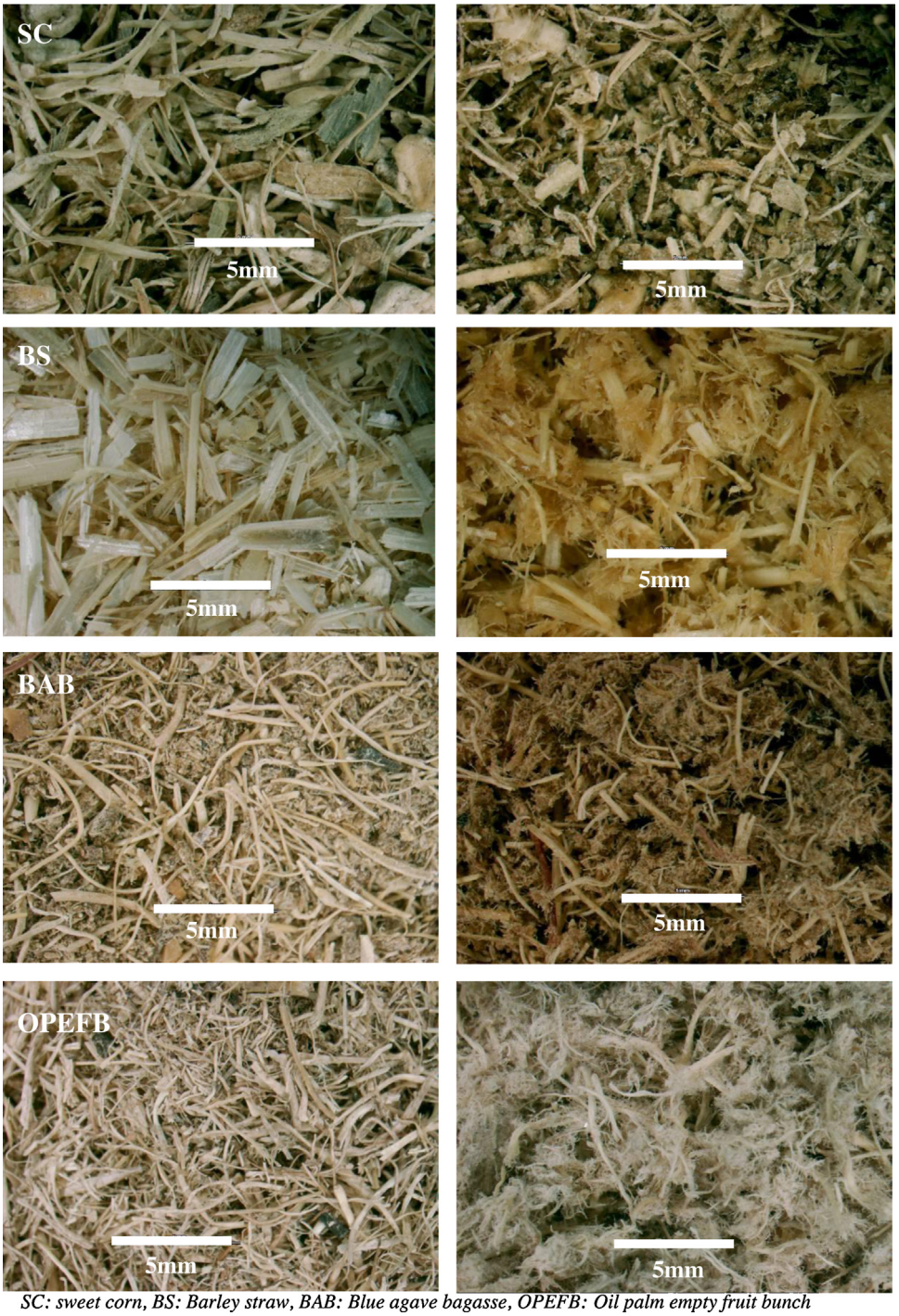


Fig. 3. Physical structure of raw and pretreated biomass selected as model feedstock.

matter after filtration is very high (55 and 67% respectively), and the amount of hot water soluble fraction still present in this extrudate (see Table 5), is very low in the case of OPEFB (2.5%).

The lignocellulosic matter is defibrated within a very short contact time, and the physical structure changes as the fibers are broken apart (Fig. 3).

The alkaline pretreatment step leads to the extraction of water-soluble components such as proteins, ash, and free sugars present in the raw biomass. Alkaline action induces solubilization of lignin and hemicelluloses as a function of sodium hydroxide concentration. Jacquemin et al., 2012 have reported effective extraction of wheat bran and straw hemicelluloses in a twin-screw extruder using alkaline extraction with a high NaOH/DM ratio (close to 50% (w/w)). With the alkaline ratio in the 7–13% range, as used in this study, the NaOH content is too low to give complete extraction of hemicelluloses and lignin. The ratio of caustic soda for OPEFB is higher than for the other biomasses because of its higher lignin content. For the four feedstocks selected only partial extraction is taking place (Tables 2 and 5).

The filtration step allows extraction of 17–23% of the dry matter except for BAB where 56% is extracted because the fine particles are driven into the filtrate. A large part of the hemicelluloses is solubilized by pretreatment (22% for OPEFB, 38% for BS, 42% for SC and 53% for BAB); however, their residual content remains high

Table 5
Composition of alkaline pretreated and bioextruded biomass.

	SC		BS		BAB		OPEFB	
	(%/DM)	(kg/kg RB)	(%/DM)	(kg/kg RB)	(%/DM)	(kg/kg RB)	(%/DM)	(kg/kg RB)
Alkaline pretreated biomass								
Dry matter	38.0	0.72	37	0.71	55.1	0.44	66.9	0.83
Ash	8	0.05	4.5	0.03	8.6	0.04	2.5	0.02
Cellulose (C)	46.4	0.33	49.5	0.32	47.2	0.21	53.1	0.44
Hemicelluloses (H)	28.8	0.21	18.8	0.20	18.5	0.08	23.0	0.19
Lignin (L)	4.8	0.03	11.8	0.12	16.4	0.07	21.4	0.18
C/(C+H+L)	58.0%		61.8%		57.5%		54.5%	
NDF reagent soluble	20.1	0.14	19.9	0.14	17.9	0.08	2.59	0.02
Organic NDF reagent soluble	15.0	0.11	15.4	0.11	9.3	0.04	0.01	<0.01
Hot water soluble	15.9	0.11	17.3	0.12	–		2.5	0.02
Bioextruded biomass								
Dry matter	28.1	0.77	23	0.76	18.8	0.45	24.5	1.08
Ash	10.4	0.08	4.9	0.03	8.3	0.04	8.1	0.09
Cellulose (C)	38.5	0.30	36.6	0.28	42.1	0.19	37.9	0.41
Hemicelluloses (H)	16.7	0.13	6.8	0.05	15.7	0.07	14.0	0.15
Lignin (L)	4.2	0.03	9.4	0.07	15.9	0.07	15.6	0.17
C/(C+H+L)	64.8%		69.3%		57.0%		56.0%	
NDF reagent soluble	40.6	0.31	47.3	0.36	26.2	0.12	32.5	0.35
Organic NDF reagent soluble	33.1	0.25	42.4	0.32	17.9	0.08	24.4	0.26
Hot water soluble	25.7	0.20	40.9	0.31	–		12.3	0.13

RB: raw biomass, DM: dry matter, SC: sweet corn, BS: Barley straw, BAB: Blue agave bagasse, OPEFB: oil palm empty fruit bunch.

(between 18.5 and 28.8% depending on biomass). The latter situation is of interest for the process, because sugars coming from the hemicelluloses can be used to enhance the potential amount of fermentable sugar from the process. Finally, lignin has not been removed entirely either (extraction of 62% for BAB but only 5% for OPEFB).

The crystallinity index measurements for the pretreated biomasses indicate that there are insignificant increases compared to the raw matter values, except in the case of BAB where the index increased from 27 to 52% (Table 4). This highlights the different nature of the biomass removed during the filtration step, and that recovered after pretreatment. The alkaline pretreatment step does not appear to significantly alter the crystallinity index, which is consistent with the observations of Karunanithy and Muthukumarappan, 2013. However, an increase in the presence of cellulose type II, whatever the biomass, revealed that the alkaline pretreatment reaches the lignocellulosic structure, and this phenomenon is similar to the mercerization described by Dinand et al., 2002.

Thus, the comparison of the effects of pretreatment on the four feedstocks revealed variable behavior for the biomasses, according to their physical structures and composition.

3.2.2. Enzyme impregnation phase

Depending on the equipment used, the extrudates obtained after alkaline pretreatment remained in the same machine (for BAB), or were introduced immediately afterwards into a second machine for enzyme impregnation (for SC, BS, and OPEFB).

During the bioextrusion step enzymes are introduced into a conveying zone (Fig. 2), and impregnation of the material is ensured by the use of a series of mechanical pressure and relaxation zones. This facilitates an intimate contact of enzyme with the biomass. Residence time in the extruders used is very short (1.5–3 min), which does not allow complete saccharification of the biomass. However, analyses of bioextrudate composition already reveal the beginning of the hydrolysis reaction in such a short time. Introduction of saccharification enzymes after the thermomechanical pretreatment of feedstocks promoted breaking down of the biomass. An increase in the amount of hot water soluble and of NDF reagent soluble matter is observed, and the organic NDF reagent soluble fraction has more than doubled, attaining 18–42% depending on the feedstock (Table 5). Overall, from 6.8% (for OPEFB) to 12.5% (for BS) of the cellulose, and 12.5% (for BAB) to 38% (for SC) of hemicelluloses have been modified. The enzymatic hydrolysis of the polysaccharide chains induces their shortening, making them solubilizable in NDF and ADF reagents. Thus they were no longer counted as cellulose or hemicelluloses with Van Soest's method. Apart from the partial modification of cell wall components, bioextrusion produced a refining of the biomass, observed by an increase in the proportion of cellulose relative to the other cell wall components (Table 5).

Continuing the saccharification after bioextrusion for 48 h at 2.5% dilution consistency, revealed that the process has produced a significant increase in the hydrolysability of the biomass resulting in a higher EH yield (Table 6). This reaches more than 55% for SC, BS and BAB, and although it tripled for OPEFB it still only reached 26%. OPEFB biomass is difficult to process using this method due to its physical structure and composition (high lignin content); its valorization would require more elaborate pretreatment conditions. The benefits of using this method have been validated for the other biomass models. Early fermentation tests showed that 9–19 g ethanol can be obtained per 100 g of bioextrudate biomasses, corresponding to 31–68% of ethanol potential calculated from the hydrolysable sugars. These results also show that the deconstruction process does not generate toxic compounds that could inhibit fermentation. Much better fermentation results are possible from a refined and optimized operation of the process. Results on the

Table 6
Glucose and xylose released after time extended to 48 h with dilution of the bioextrudate to 2.5% consistency (in percentage of sugars released from the dry matter).

(%/DM)	SC	BS	BAB	OPEFB
Glucose (% of DM)	38	38	49.5	16.4
Xylose (% of DM)	19	17	19.4	9.6
EH yield (% of DM)	57	55	69	26

DM: dry matter, SC: sweet corn, BS: Barley straw, BAB: Blue agave bagasse, OPEFB: oil palm empty fruit bunch.

changes made to the process will be the scope of future publications from our group.

4. Conclusion

A new process for the deconstruction of lignocellulosic biomasses, consisting of a thermo-mechano-chemical action combined with an enzymatic hydrolysis biological action, in a twin-screw extruder, has been developed. The results obtained in this study prove the effectiveness of this new process.

The alkaline thermo-mechano-chemical pretreatment allows the destruction of the wall polymers, although the results differ depending on the processed biomass. Hemicelluloses are partially solvated and extracted by the pretreatment (between 22% and 53% are extracted), and delignification is more or less pronounced (extraction of up to 62% of lignin for BAB and only 5% for OPEFB). Finally, the alkaline pretreatment produces a biomass with increased hydrolysability.

Saccharification begins during bioextrusion, and a large part of the biomass became soluble in the NDF reagent (between 26 and 47%). Thus, between 7 and 13% of the cellulose, and 12 and 75% of hemicelluloses, were no longer recognized as cellulose or hemicelluloses by Van Soest's method. However, extruder residence time is too short to obtain significant saccharification directly, and needs to be extended after bioextrusion. EH Yield of the biomass at the end of the process reaches more than 55% except for OPEFB (26%). This process, coupling alkaline pretreatment and bioextrusion, shows enormous potential that deserves further studies to bring an optimized pretreatment step for ethanol production.

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