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# Suitability assessment of a continuous process combining thermo-mechano-chemical and bio-catalytic action in a single pilot-scale twin-screw extruder for six different biomass sources

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## H I G H L I G H T S

- Adaptation of lignocellulosic biomass deconstruction process on pilot scale extruder.
- Combination of alkali pretreatment and biocatalytic action in extruder.
- Validated of the process on six different lignocellulosic biomasses.

## A B S T R A C T

A process has been validated for the deconstruction of lignocellulose on a pilot scale installation using six types of biomass selected for their sustainability, accessibility, worldwide availability, and differences of chemical composition and physical structure. The process combines thermo-mechano-chemical and bio-catalytic action in a single twin-screw extruder. Three treatment phases were sequentially performed: an alkaline pretreatment, a neutralization step coupled with an extraction-separation phase and a bioextrusion treatment. Alkaline pretreatment deconstructed the wall polymers after just a few minutes and allowed the initial extraction of 18–54% of the hemicelluloses and 9–41% of the lignin. The bioextrusion step induced the start of enzymatic hydrolysis and increased the proportion of soluble organic matter. Extension of saccharification for 24 h at high consistency (20%) and without the addition of new enzyme resulted in the production of 39–84% of the potential glucose.

### Keywords:

Lignocellulosic biomass  
Twin-screw extruder  
Alkaline pre-treatment  
Enzymatic hydrolysis  
Bioextrusion

## 1. Introduction

The production of both new feedstock and clean energy from biomass is faced with the problem of whether to promote food or energy. In the context of sustainable development and environmental protection, it is essential to turn to nonedible feedstocks such as agricultural residue, forest residue, industrial waste, dedicated energy crops and municipal solid waste (Ragauskas et al., 2006). Cellulose, hemicelluloses and lignin in the plant cell wall are tightly associated. Classical production processes to generate monomers of interest, including enzymatic hydrolysis and fermentation, work poorly on the lignocellulosic biomass. The conversion of renewable biomass to fuels and chemicals requires the

deconstruction of lignocellulose assembly (Himmel et al., 2007). Increasing the efficiency of lignocellulosic conversion to sugars presents a major challenge. There are many research groups working on new processes for obtaining fuels from biomass and several reviews on developing pretreatment strategies have been published (Mood et al., 2013; Singh et al., 2014; Ravindran and Jaiswal, 2015).

The types of biomass to be used depends on the desired area of application. In the field of energy, these are sustainable, accessible and available materials which are rich in cellulose. The most studied biomass for the production of energy has been sugarcane bagasse (SCB), of which the estimated annual dry mass production was ~279 million metric tons (MMT) in 2011 (bagasse and leaves) (Chandel et al., 2012). Cardona et al. published a review in 2010 on various pretreatment processes to produce bioethanol using this material and proposed future strategies (Cardona et al., 2010). SCB has since been used as a model to develop the important steps

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of the process to produce bioethanol. Rocha et al. (2012) studied the efficiency of a pretreatment process combining steam explosion and alkaline delignification reactions. Zhu et al. (2012) pretreated SCB with  $\text{NH}_4\text{OH}-\text{H}_2\text{O}_2$  and ionic liquid resulting in efficient hydrolysis and bioethanol production. Maryana et al. (2014) evaluated the impact of alkaline pretreatment on the chemical composition and structure of SCB. Sambusiti et al. (2015) studied the effect of different milling methods on the physicochemical composition, enzymatic hydrolysis, bioethanol production and energy efficiency of the process.

Oil palm empty fruit bunches (OPEFB) is another biomass feedstock that has been extensively studied for potential second generation bioethanol production, in part because of its availability (23.4 million tons produced in Indonesia in 2011: Millati et al., 2014). It has been subjected to most conventional pretreatments: AFEX (Lau et al., 2010), Acid hydrolysis (Millati et al., 2011), alkaline pretreatment (Han et al., 2011), and aqueous ammonia pretreatment (Jung et al., 2011). More recently, Sudiyani et al. (2013) have obtained promising results using an integrated process that included alkaline pretreatment at the pilot scale. Chiesa and Gnansounou (2014) compared dilute acid and dilute alkali pretreatment and showed that dilute alkali pretreatment performed poorly due to the significant lignin content of the OPEFB. Kristiani et al. (2015) studied the effect of combining alkaline treatment with an irradiation pretreatment process using an electron beam machine. This combination affected the structure of OPEFB by decreasing the lignin content and changing the crystallinity index.

Wood residues are another potential energy source. Eucalyptus globulus is one of the most commercially important hardwood species. In 2004, there were about 2.5 million hectares planted worldwide (Catry et al., 2013). Eucalyptus production and processing generates a large amount of wood residues, such as sawmill residues or bark and branches currently left in the field. The residues can reach 30% of the total harvested biomass (15–25 ton/ha/year) Lima et al. (2013). Eucalyptus wood and bark are harder and denser than grass or cereal biomass, and are resistant to microbial and enzymatic action as a result of their higher lignin content. Zhu and Pan (2010) has presented a comprehensive discussion of the key technical issues of woody biomass pretreatment including: dilute acid, acid-catalyzed steam explosion, organosolv, sulfite, and alkaline pretreatment. Work on eucalyptus is ongoing to improve the enzymatic accessibility of the biomass such as: hydrothermal, dilute acid, and alkaline pretreatment (de Carvalho et al., 2015), hydrothermal microwaves using acidic ionic liquid as catalyst (Xu et al., 2015), and steam explosion processing (Romaní et al., 2013).

Other less studied sources of biomass can serve as a potential energy source. Among these is vineyard pruning (VP). Pruning of grape trees generates high quantities of lignocellulosic biomass (Velazquez-Martí et al., 2011): about 21 million tons of pruning waste are produced each year (Argun and Onaran, 2015). Buratti et al. (2014) pretreated it using steam explosion to produce ethanol and Argun and Onaran (2015) studied its delignification using alkaline peroxide.

Agave bagasse is a residue that accumulates during the production of alcoholic beverages from plants of the agavaceae family. It offers a potential sustainable resource that was estimated to be produced at a rate of around 360 thousand dry tons per year (Caspeta et al., 2014). Nguyen (2014) deposited a patent on a process for producing ethanol using acid-catalyzed steam pretreatment of agave bagasse. Perez-Pimienta et al. (2015) thoroughly characterized agave bagasse following ionic liquid pretreatment.

Sweet corn residue is another potential source of biomass for energy production. The worldwide production of sweet corn was about 2.9 million tons of grain in 2012 (Hansen, 2013). The produc-

tion of sweet corn residue can be estimated to be approximately 6 million tons per year because its weight is twice that of the grain. This biomass is mostly used for forage for ruminant animals and has been little studied for energy production. Its use as a source of sugar for energy production has been tested in previous studies using alkaline pretreatment followed by bio-catalytic hydrolysis in a twin screw extruder (Vandenbossche et al., 2014b, 2015).

Increasing the solid concentration of biomass, and thereby decreasing the volume to be treated, could improve the conversion process and lower the costs. However, this increases the viscosity of biomass slurries, making mixing and conveying operations more difficult. Among the processes used to carry out pretreatment with a minimum number of steps, twin-screw extrusion technology offers many advantages and permits working with high solid concentrations. It produces a high shear, rapid heat transfer, and effective and rapid mixing in a continuous operation, with good adjustability of the treatment steps.

Twin-screw extrusion can be used to pretreat different types of biomass for the production of sugars. Several authors have reported this type of application (Vandenbossche et al., 2014a; Zheng and Rehmann, 2014). Karunanithy and Muthukumarappan (2013) provide an overview of the combination of extrusion with alkaline pretreatment, including the factors that influence extruder and feedstock parameters and an evaluation of the pretreatment efficiency. A continuous process combining alkaline thermo-mechano-chemical pretreatment, followed by the injection of enzymes into the twin-screw extruder, called “bioextrusion” was developed and tested on different biomass sources such as sweet corn residue (SC), blue agave bagasse, OPEFB as a residue from palm oil manufacture, and barley straw (Vandenbossche et al., 2014b). This new process results in excellent mixing of the enzymes with the pretreated biomass at high concentrations, and allows saccharification to begin during bioextrusion (Vandenbossche et al., 2015).

This continuous process had been developed and tested at the pilot scale in a single extrusion in this study using different types of biomass.

## 2. Materials and methods

### 2.1. Material

#### 2.1.1. Feedstocks

Dehydrated sweet corn (*Zea mays* L. *saccharata*) co-products (SCC) were obtained from industrial corn grain canneries and were provided by SAREL Soupro+ (Castelmoron sur Lot, France). They were milled using a hammer mill fitted with a 6 mm grid.

SCB came from Brazil and was provided by EMBRAPA. They were milled using a hammer mill fitted with a 6 mm grid.

Sawdust of *Eucalyptus grandis* (SE) came from Uruguay and were provided by the Instituto Nacional de Investigación Agropecuaria (INIA). They were milled using a hammer mill fitted with a 6 mm grid.

VP came from Chile and were provided by the Instituto Nacional de Investigación Agropecuaria (INIA). 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.

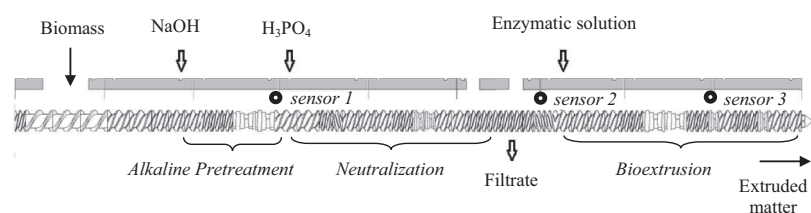


Fig. 1. Schematic diagram of the modular barrel of the EV 53 twin-screw extruders.

BAB and OPEFB had to be ground finer. Milled with a 6 mm grid, they formed a fibrous network which posed problems to supply the extruder with hopper system used.

### 2.1.2. Extrusion

The extrusion process was performed using a twin-screw extruder (Evolum 53, Cletral, France), composed of 9 modules with a length of 212.60 mm each. The extruder is configured to combine three different treatments: alkaline pretreatment, neutralization and filtration of the matter and bioextrusion (Fig. 1). The screw diameter is 52.45 mm. Four types of screws were used: a trapezoidal double-thread screw used for the feeding zone, conveying double-thread, bilobed paddle, and reversed double-thread screws used to produce transport, mixing, and shearing effects, respectively, along the different zones of the process. The screw profile was the same for all the biomasses except for sweet corn which required a longer reverse screw (Table 1). Modules were thermostated by a heating band and cooled by water circulation. A filter section consisting of six hemispherical dishes with conical holes (1 mm entry, 2 mm exit) was used on module 6 to enable the filtrate to be collected. Feedstocks were fed into the extruder's first module using a weigh belt feeder model number SWB-300-N (K-tron). Three piston pumps (DKM Super K Camp 112/12, DKM Super MD-PP-63 and DKM K202 P32) were used to inject, respectively, an alkaline solution of sodium hydroxide (NaOH), an acid solution of phosphoric acid ( $H_3PO_4$ ) and an enzymatic solution. The operating conditions were based on conditions previously developed for a process using two extruders (Vandenbossche et al., 2014b) and adapted to a single extruder (Tables 2 and 3).

### 2.1.3. Enzyme cocktails for bioextrusion

The bioextrusion phase was conducted using three different hydrolytic enzyme cocktails, depending on the biomass, that were introduced in module 7. The enzyme solution was prepared by mixing cellulase and hemicellulase preparations at a protein content ratio of 9:1, in 200 mM citrate buffer (except for sweet corn in which the buffer was 300 mM), pH 4.8. Cellulase and hemicellulase cocktails were: saccharised C6 (advanced enzyme Technologies – India) plus viscozyme for sweet corn and Cellic Ctec plus viscozyme for the other types of biomass. Viscozyme and Cellic Ctec were kindly provided by Novozymes A/S (Denmark).

To evaluate the efficiency of glucose production of the extruded matter following the bioextrusion phase, water was added to the bioextrudate to obtain an approximate consistency of 20%. The mixture was then incubated in a stirred tank reactor between 16 and 24 h depending on the biomass. Hydrolysis was carried out at 50 °C at a pH of 5 regulated by ortho-phosphoric acid.

Table 1  
Adaptation of the screw profile depending on the biomasses.

Material	Sweet corn	Sugar cane bagasse	Eucalyptus	Vineyard pruning	Blue agave bagasse	OPEFB
Additional reverse screw in module 4	No	Yes	Yes	Yes	Yes	Yes
Length of the reverse screw in module 7 (mm)	78.67	52.45	52.45	52.45	52.45	52.45

## 2.2. Analytical methods

### 2.2.1. Dry matter and parietal compounds

Moisture content was determined according to the French standard NF V 03-903, and mineral content according to the French standard NF V 03-322. An estimation of the three parietal constituents (cellulose, hemicelluloses, and lignin) contained in the solids, was performed using the ADF-NDF method of Van Soest and Wine (1968). All determinations were carried out in triplicate and the standard deviation was less than 1.5% for all measurements.

An estimation of the hot water-soluble components was made by measuring the mass loss of the test sample after 1 h in boiling water. This method has been adapted using standard TAPPI 204 cm-97 on the Fibertec Tecator M1017 apparatus. All determinations were carried out in duplicate.

### 2.2.2. Glucose analysis

Glucose released after incubation of the extrudate in a stirred tank reactor at 50 °C between 16 and 24 h were measured using HPLC (waters 2695 liquid chromatograph with refractive index detector and aminex HPX-87H column).

### 2.2.3. Enzymatic hydrolyzability

Enzymatic hydrolyzability was determined by enzymatic hydrolysis in 50 mM citrate phosphate buffer (pH 4.6) using Advanced enzymes (2.5%/DM substrate) at 50 °C for 48 h. It was calculated as the percentage of reducing sugars released by enzymatic hydrolysis, relative to dry matter, or to the theoretical C6 content. Reducing sugars were determined using the DNS method (Miller, 1959).

## 3. Results and discussion

The process of deconstruction of lignocellulosic plant material, including bioextrusion, has been developed using one or two consecutive extruders at the laboratory scale (Vandenbossche et al., 2014b). Industrialization of the process requires adapting the twin-screw extrusion technology to integrate all process operations into a single step twin-screw extrusion device at a higher scale. The three different steps of the deconstruction process are carried out in a single EV 53 extruder able to achieve a feed rate of 100–200 kg/h (Fig. 1).

### 3.1. Biomass

The adaptability of the process to accommodate different types of biomass has been tested using six different feedstocks: sweet

**Table 2**  
Operating conditions.

Material	Sweet corn	Sugar cane bagasse	Eucalyptus	Vineyard pruning	Blue agave bagasse	OPEFB
Granulometry (mm)	6	6	6	6	2	2
S <sub>s</sub> (rpm)	200	200	200	200	100	175
Q <sub>s</sub> (kg/h)	33.0	20.0	20.0	20.0	20.0	20.0
FR	0.16	0.10	0.10	0.10	0.20	0.11
Q <sub>NaOH</sub> (kg/h)	30.0	22.0	22.0	22.0	23.0	40.0
[NaOH] (%) (m/m)	10.0	7.2	7.2	7.2	7.0	10.0
Q <sub>H3PO4</sub> (kg/h)	90.0	53.0	53.0	53.0	57.0	100.0
[H <sub>3</sub> PO <sub>4</sub> ] (%) (m/m)	3.5	3.0	3.0	3.0	3.0	4.0
Q <sub>t</sub> (kg/h)	17.0	10.0	10.0	10.0	10.2	33.3
Q <sub>ez</sub> (kg/h)	0.42	0.48	0.48	0.48	0.48	1.00
Q <sub>ext</sub> (kg/h)	104.0	49.6	45.1	49.0	61.0	61.0
Q <sub>fil</sub> (kg/h)	61.0	58.8	62.8	60.6	43.0	130.0
DM <sub>ext</sub> (%)	27.2	38.0	40.1	34.1	32.7	27.2
NaOH/DM (%)	10.0	8.1	8.1	8.1	9.0	21.3
ez/DM (%)	1.5	2.5	2.5	2.5	2.6	5.3
L/S <sub>b</sub>	0.9	1.0	1.0	1.0	1.1	1.6
L/S <sub>a</sub>	3.2	3.2	3.2	3.2	3.6	5.0
L/S <sub>ez</sub>	3.5	3.4	3.4	3.4	3.8	5.5

S<sub>s</sub> is the screw rotation speed (rpm); Q<sub>s</sub> is the material feed rate (kg/h); FR is the extruder filling ratio. Q<sub>NaOH</sub>, Q<sub>H3PO4</sub>, Q<sub>t</sub> and Q<sub>ez</sub> are the inlet flow of NaOH, H<sub>3</sub>PO<sub>4</sub>, enzymatic solution and enzyme expressed as protein equivalent, respectively; [NaOH] and [H<sub>3</sub>PO<sub>4</sub>] are the concentration of the reagent solution; Q<sub>ext</sub> and Q<sub>fil</sub> are the flow rate of the extrudate and filtrate (kg/h); DM<sub>ext</sub> is the dry matter of the extrudate; NaOH/DM and ez/DM are the reagent to material dry matter ratio (%); L/S<sub>b</sub>, L/S<sub>a</sub>, L/S<sub>ez</sub> are the liquid/solid ratio of the alkaline pre-treatment phase, the neutralization phase, and the bioextrusion, respectively.

**Table 3**  
Energy data.

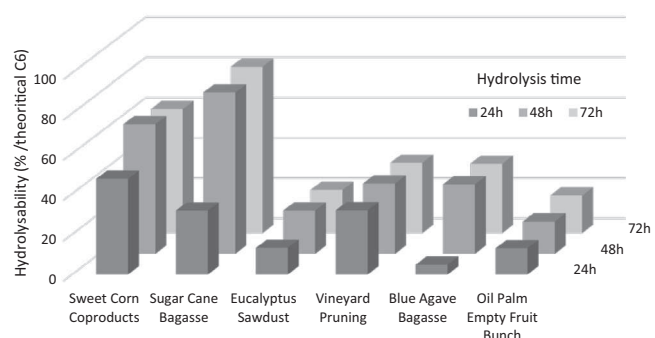
Material	Sweet corn	Sugar cane bagasse	Eucalyptus	Vineyard pruning	Blue agave bagasse	OPEFB
SME (Wh.kg <sup>-1</sup> )	88	408	445	207	288	243
STE (Wh.kg <sup>-1</sup> )	170	25	32	131	102	54
S <sub>s</sub> (rpm)	200	200	200	200	100	175
T <sub>s</sub> (°C)	22/50/100/100/55/55/ 35/35/35	25/50/75/75/50/-/ 25/25/25	25/50/75/75/50/-/ 25/25/25	25/50/75/75/50/-/ 25/25/25	22/50/75/75/50/50/ 35/35/35	22/50/100/100/55/55/ 35/35/35
T <sub>r</sub> (°C)	20/50/100/100/55/55/ 41/40/37	25/50/81/78/51/69/ 34/39/26	25/50/83/83/55/75/ 49/36/27	25/48/70/75/58/60/ 25/25/24	20/50/80/82/55/55/ 41/40/37	22/49/99/99/58/78/35/ 35/35
T <sub>p</sub> (°C) (sensor 1/2/3)	90/56/42	84/85/40	85/89/45	69/62/33	80/68/43	97/104/47
P <sub>p</sub> (bar) (sensor 1/2/3)	1.1/1.7/0.1	-	-	-	0.3/7.1/0.4	0.1/18.5/0.5

SME and STE are the specific mechanical and thermal energy consumed by the motor of the EV53 extruder per unit weight of solid matter (Wh/kg). S<sub>s</sub> is the screw rotation speed (rpm); T<sub>s</sub> and T<sub>r</sub> are the setpoint and the real temperature of each module, respectively. T<sub>p</sub> and P<sub>p</sub> are the temperature and the pressure in contact with the matter measured by the probes (1, 2 and 3), respectively.

corn coproducts (SCC), sugar cane bagasse (SCB), sawdust of eucalyptus (SE), vineyard pruning (VP), blue agave bagasse (BAB), and oil palm empty fruit bunch (OPEFB). These sources of biomass were selected for their sustainability, accessibility, and worldwide availability, as well as their differences in chemical composition and physical structure. The extent to which the raw materials could be hydrolyzed were first tested. The results are summarized in Fig. 2. The release of sugars by enzymatic hydrolysis after 24 h was almost zero from BAB, 5% to 7% from OPEFB, barley straw and ES, near 12% for VP and SCB, and 18% for SCC. This result suggests that the cellulose of SCC, SCB, and VP have the best enzymatic accessibility. SCB had the highest level of sugar release (32%), followed by CSC, when the hydrolysis time was extended; sugar release from OPEFB was only 7–9%. SCC and SCB were the most easily hydrolyzable feedstock while OPEFB was the most resistant material in the absence of pretreatment. Altogether, these data show that the organization and composition of their tissues affects the extent to which different types of biomass can be hydrolyzed. The enzymatic hydrolyzability (based on the cellulose content of each raw material) was inversely correlated with the lignin content. Thus, lignin appears to reduce the hydrolyzability of cellulose proportional to its content in the biomass, except for SCB (Fig. 3). This effect of lignin is in accordance with previously published work (Berlin et al., 2006; Öhgren et al. 2007 and Pan, 2008). But the case of SCB shows that this is only a trend and that other

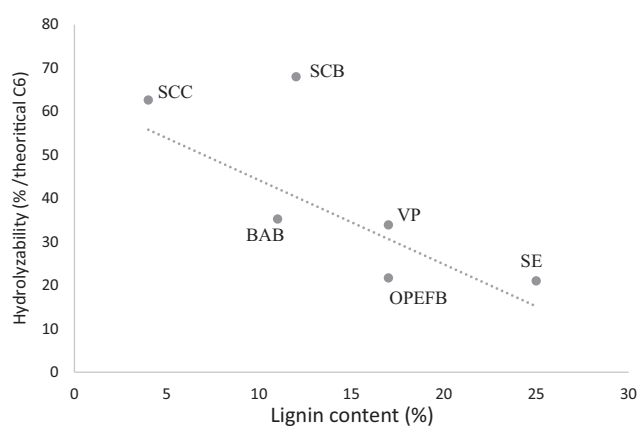
parameters are involved. It shows that lignin content is not the only parameter to impact the hydrolyzability. The different behavior of SCB may be due to the industrial pretreatment to which it was already been subjected. The water-soluble content, non-parietal compounds and hemicelluloses may also affect the hydrolyzability of the cellulose.

The differences in enzymatic accessibility for each source of biomass suggests that it will be necessary to adapt the operating conditions of the process depending on the material used.



**Fig. 2.** Enzymatic hydrolyzability of the raw material for different hydrolysis times.





**Fig. 3.** Correlation between enzymatic hydrolyzability and lignin content of the raw material.

### 3.2. Operating conditions

The differences in the physical structure of the various sources of biomass required adaptation of the operating conditions. The materials were ground to a particle size that allowed control of the feed rates (2 mm for BAB and OPEFB, which consist of a fibrous network making it impossible to introduce it into the densifier at a higher particle size and 6 mm for the other lignocellulosic materials). The flow-rate for the biomasses was 33 kg/h for SC and 20 kg/h for the other biomass sources because of limited available material, but could be largely increased for this type of machine.

The operating conditions were first determined for the alkaline pretreatment, neutralization, solid-liquid separation and bioextrusion within the single EV53 extruder using SC and then adapted to the other biomass sources. The operating conditions were determined step by step. The material feed rate and the flow of NaOH was first adjusted to the desired value. When the system was stable,  $H_3PO_4$  was introduced at a flow-rate to provide efficient neutralization. The screw-rotation speeds were set to a value that ensured good stability and efficient solid-liquid separation at a regular filtration rate for each source of biomass. After 15 min, the enzymatic solution was added until the desired rate was achieved.

The biomass sources other than SC required more extensive thermomechanical destructuring in the alkaline pretreatment zone, because they are more fibrous and lignified than SC. The profile in the alkaline destructuring zone determined for SC was slightly modified (Fig. 1) by adding a reverse screw in module 4 (Table 1) to accommodate the other sources of biomass.

Solid-liquid separation is facilitated by the fibers in the biomass due to the formation of a hard “dynamic plug”. In this study, the additional sources of biomass are considerably richer in fibers than SC. Thus, the length of the reverse screw in module 7 was reduced (Table 1) to avoid generating a “dynamic plug” which was too hard; thus avoiding excessive self-heating and mechanical stress which could cause the machine to lockup.

The alkaline ratio (NaOH/DM) was set between 8% and 10%, except for the OPEFB for which it was more than doubled (21.3%). This value was chosen because of the high lipid content of OPEFB, which increases the rate of alkaline reagent consumption, and the results of previous experiments using BC45 and BC21 extruders (Vandenbossche et al., 2014b).

The enzyme ratio (ez/DM) was initially set to 1.5% for SC. This was increased to 2.5% for the other biomass sources, except for OPEFB for which it was further increased to 5.3%.

The bioextrusion process was stable and exhibited good temperature control of the modules for all of the tested biomass sources (Table 3).

The set temperature for the pretreatment zone was set between 75 and 100 °C depending on the biomass source. The temperatures for the other zones were adjusted to result in a temperature for the material between 40 and 50 °C in the bioextrusion zone to avoid degrading the enzymes. Self-heating occurred in the compression zone during the filtering step (Sensor 2) for all fibrous materials. This correlated with a lower STE, higher SME, and a substantial increase of the measured pressure when processing the BAB and OPEFB (Table 3).

### 3.3. Extraction and filtration yield

The extrudate dry matter before injection of the enzymatic solution ( $DM_{\text{pret,ext}}$ ) reflects the quality of the filtration. It may serve as an indicator of the pressing efficiency of the lignocellulosic material after neutralization in the extruder. The pressing efficiency was excellent for all biomasses (*i.e.* a  $DM_{\text{pret,ext}}$  higher than 45%) except for sweet corn which is less fibrous and had a  $DM_{\text{pret,ext}}$  of only 30.5% (Table 4).

When the alkaline ratio was less than 10% (NaOH/DM), the filtration yield of organic matter was between 20% and 30% and the extraction yield between 9% and 20%. The extraction yield reached 44% for OPEFB, for which the proportion of sodium hydroxide was 21.3%.

### 3.4. Composition of the extrudate before and after the pretreatment phase

The content of the different biomass sources was compared before and after pretreatment (including the filtration phase). Pretreatment by alkaline attack, neutralization, and liquid-solid separation, without the addition of enzyme into the extruder did not substantially change the water-soluble organic content of the extrudates compared to the starting material. This is due to the filtration step which directs the soluble compounds to the filtrate (Table 5). Pretreatment mostly affected by the hemicellulose content which decreased between 18% and 54% depending on the initial hemicellulose content of the biomass and the NaOH ratio used in the process. Lignin was less affected, except for OPEFB for which the NaOH ratio was much higher. The observed loss of cellulose following this treatment step was mainly due to the transfer of fine particles to the filtrate. The partial solubilization of the hemicelluloses and, for some biomass sources, the lignin, resulted in a substantial increase in the proportion of cellulose found in the insoluble fiber. The change in the proportion of the parietal constituents confirms that destructuring of the lignocellulosic fibers took place in the pre-treatment zone.

### 3.5. Composition of the extrudate before and after the bioextrusion phase

The material composition of the extrudate was compared before and after the bioextrusion phase. There was a highly substantial increase in the proportion of water-soluble organic matter that resulted from the process (Table 5). The proportion of the water-soluble organic matter increased two to threefold for BAB, OPEFB and VP, fivefold for SCB, and 13-fold for SE.

The effectiveness of the bioextrusion phase varied for each biomass source. The yield of recovered cellulose in the bioextrudate was high for all co-products (74–89%) except for SE (60%). The loss of cellulose in this step results from its partial enzymatic hydrolysis. Cellulose decreased by 12% for SCC, 13% for VP, 19% for SCB, 27% for BAB and 31% for SE. There was also further solubilization of the hemicelluloses and lignin. There was a high level of solubilization of the hemicelluloses of SCB (54%) whereas those for the

**Table 4**

Flows and extraction yields of the process.

Material	Sweet corn	Sugar cane bagasse	Eucalyptus	Vineyard pruning	Blue agave bagasse	OPEFB
$Q_{ext}$ (kg h <sup>-1</sup> )	104.6	49.6	45.1	49.0	60.7	61.4
DM <sub>pret.ext</sub> (%)	30.5	53.3	56.3	50.7	45.6	53.5
DM <sub>ext</sub> (%)	27.2	38.0	40.1	34.1	32.7	27.6
% solid recovery (g/100 g of dry initial biomass)	95.4	96.6	92.9	85.6	106.8	90.4
$Q_{fil}$ (kg h <sup>-1</sup> )	61.0	58.8	62.8	60.6	42.5	130.0
DM <sub>fil</sub> (%)	11.1	7.8	5.7	9.4	10.5	11.5
OM <sub>fil</sub> (%/DM)	62.7	56.6	50.2	63.1	65.3	53.7
Filtration yield (g DM/100 g DM of dry initial biomass)	22.7	23.4	18.5	29.3	23.9	79.9
Extraction yield in filtrate (g OM/100 g OM of dry initial biomass)	14.6	13.5	9.3	19.5	17.3	44.4

$Q_{ext}$  and  $Q_{fil}$  are the flow rates of the extrudate and filtrate (kg/h); DM<sub>ext</sub> and DM<sub>fil</sub> are the dry matter of the extrudate and filtrate, respectively; DM<sub>pret.ext</sub> is the dry matter of the extrudate before injection of the enzymatic solution; OM<sub>fil</sub> is the organic matter in the filtrate.

other biomass sources were much lower: BAB (16%), VP (17%) OPEFB (12%) and SE (11%). Enzymatic hydrolysis of the hemicelluloses was significantly higher for those biomass sources for which the solubilization of hemicelluloses by alkaline destructuring was also high.

Although some lignin was also solubilized by enzymatic action (40% for VP), the extraction yield remained low for most biomass sources (5–13%). The fraction of solubilized lignin may correspond to that associated with the hemicelluloses which were hydrolyzed and solubilized by the action of enzymes.

**Table 5**

Mass balance during the process expressed for 100 kg of dry biomass.

Material	Starting material Quantity (kg)	Pretreated <sup>*</sup>		Bioextruded <sup>**</sup>	
		Quantity (kg)	Yield (%)	Quantity (kg)	Yield (%)
<i>Sweet corn</i>					
Dry matter	100	89	–	95	–
Cellulose	39	33	85	29	74
Hemicelluloses	36	26	72	15	42
Lignin	4	3	75	2	50
Total insoluble fiber	79	78	87	56	71
Soluble organic matter	13	14	–	31	–
<i>Sugar cane bagasse</i>					
Dry matter	100	89	–	97	–
Cellulose	47	48	100	39	83
Hemicelluloses	35	22	63	10	28
Lignin	12	11	91	10	83
Total insoluble fiber	94	82	87	59	63
Soluble organic matter	5	4	–	25	–
<i>Eucalyptus</i>					
Dry matter	100	85	–	93	–
Cellulose	60	52	86.5	36	60
Hemicelluloses	11	9	82	8	73
Lignin	25	20	80	19	76
Total insoluble fiber	96	81	84	63	65
Soluble organic matter	2	3	–	24	–
<i>Vineyard pruning</i>					
Dry matter	100	78	–	86	–
Cellulose	43	37	86	32	74
Hemicelluloses	26	12	46	10	38
Lignin	17	15	88	9	53
Total insoluble fiber	86	64	74	51	59
Soluble organic matter	10	9	–	26	–
<i>Blue agave bagasse</i>					
Dry matter	100	94	–	101	–
Cellulose	41	44	107	32	78
Hemicelluloses	24	12	50	10	41.5
Lignin	11	8	73	7	63.6
Total insoluble fiber	76	64	84	49	64
Soluble organic matter	10	11	–	36	–
<i>OPEFB</i>					
Dry matter	100	74	–	90	–
Cellulose	44	38	86	38	86
Hemicelluloses	27	16	59	14	52
Lignin	17	10	59	9	53
Total insoluble fiber	88	64	72	61	69
Soluble organic matter	9	6	–	21	–

<sup>\*</sup> Pretreated: extruded without adding enzymatic solution.

<sup>\*\*</sup> Bioextruded: extruded after adding enzymatic solution.



**Table 6**

Hydrolysis yields for the bioextruded biomass sources incubated for 24 h in batches with a consistency of 20%.

Biomass	Sweet corn	Sugar cane bagasse	Eucalyptus	Vineyard pruning	Blue agave bagasse	OPEFB
Consistence (%)	20	20	20	20	20	20
Reaction time (h)	20	24	24	24	24	16
Ratio enzyme/dry matter (mg protein/g dry extrudate)	52	20	20	20	20	53
Glucose production yield (g/100 g DM bioextrudate)	26	25	15	20	33	18
Glucose production yield (%/theoretical C6)	84	63	39	53	79	44
Glucose production yield (g/100 g DM biomass)	25	24	14	18	35	17

The destructuring process of SCB, BAB, SCC and VP in one step in the EV53 twin-screw extruder is adapted to the disintegration and refining of lignocellulosic fibers. Between 60% and 70% of the hemicelluloses could be extracted following exposure to less than 10% sodium hydroxide for a few minutes, and enzyme levels of 2.5%. The yield for lignin solubilization was 40–50% for highly lignified products (BAB and VP) and almost 20% for the less lignified SCB. The yields of solubilized lignin and hemicelluloses were also high (near 50%) for pinzote, but required doubling of the amount of sodium hydroxide and enzyme levels. The yields were low (less than 30% of the hemicelluloses and 25% of the lignin were solubilized) for SE, which contains very little hemicellulose. This could be improved by using a higher amount of sodium hydroxide or enzymes.

In all cases, the hydrolyzability of the materials was improved compared to that of the raw co-products (Table 6). Enzymatic hydrolysis can be carried out directly without adding additional enzymes to the bioextrudates at high consistency (20%) for a limited time (24 h).

The glucose yield depended on the physical structure and composition of the treated biomass sources: it was 79–84% for BAB and SC, but only 39–44% for SE and OPEFB. The poor results obtained with SE and OPEFB indicate that these materials are highly resistance to enzymatic hydrolysis, already observed for SE by Zhu and Pan (2010) and OPEFB in a previous study (Vandenbossche et al., 2014b). These biomass sources would require more extensive pretreatment to improve enzyme accessibility.

This method, which combines alkali pretreatment, neutralization and extraction–separation, and enzyme treatment using a cellulase, in a single operation, by twin-screw extrusion, is mostly suitable for the saccharification of cellulose. However, the operating conditions will need to be adjusted depending on the characteristics of each biomass source. This process represents an important advance in the first steps toward the biorefining of lignocellulosic materials. One potential application of this process is the fermentation of glucose to ethanol but other uses could also be considered.

#### 4. Conclusions

The process of the deconstruction of lignocellulosic plant material, including alkali pretreatment, neutralization, extraction–separation and bioextrusion, has been developed using a pilot scale EV53 extruder and validated using six different lignocellulosic biomass sources. The bioextrusion step allows introduction of the enzymes to the heart of the material enabling the start of enzymatic hydrolysis. The saccharification at high consistency without the addition of new enzyme can be extended in a stirring reactor. These results verify the effectiveness and high adaptability of this process. However, harsher pretreatment conditions will be required for the most resistant biomass sources.

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#### References

- Argun, H., Onaran, G., 2015. Delignification of vineyard pruning residues by alkaline peroxide treatment. *Ind. Crops Prod.* 74, 697–702.
- Berlin, A., Balakshin, M., Gilkes, N., Kadla, J., Maximenko, V., Kubo, S., Saddler, J., 2006. Inhibition of cellulase, xylanase and  $\beta$ -glucosidase activities by softwood lignin preparations. *J. Biotechnol.* 125, 198–209.
- Buratti, C., Barbanera, M., Lascaro, E., 2014. Ethanol production from vineyard pruning residues with steam explosion pretreatment. *Environ. Prog. Sustainable Energy* 34, 802–809.
- Cardona, C.A., Quintero, J.A., Paz, I.C., 2010. Production of bioethanol from sugarcane bagasse: status and perspectives. *Bioresour. Technol.* 101, 4754–4766.
- Caspeta, L., Caro-Bermúdez, M.A., Ponce-Noyola, T., Martínez, A., 2014. Enzymatic hydrolysis at high-solids loadings for the conversion of agave bagasse to fuel ethanol. *Appl. Energy* 113, 277–286.
- Catry, F., Moreira, F., Tujeira, R., Silva, J., 2013. Post-fire survival and regeneration of *Eucalyptus globulus* in forest plantations in Portugal. *For. Ecol. Manage* 310, 194–203.
- Chandel, A.K., da Silva, S.S., Carvalho, W., Singh, O.V., 2012. Sugarcane bagasse and leaves: foreseeable biomass of biofuel and bio-products. *J. Chem. Technol. Biotechnol.* 87, 11–20.
- Chiesa, S., Gnansounou, E., 2014. Use of Empty fruit bunches from the oil palm for bioethanol production: a thorough comparison between dilute acid and dilute alkali pretreatment. *Bioresour. Technol.* 159, 355–364.
- De Carvalho, D.M., Sevastyanov, O., Penna, L.S., da Silva, B.P., Lindström, M.E., Colodette, J.L., 2015. Assessment of chemical transformations in eucalyptus, sugarcane bagasse and straw during hydrothermal, dilute acid, and alkaline pretreatments. *Ind. Crops Prod.* 73, 118–126.
- Han, M., Kim, Y., Kim, S.W., Choi, G.W., 2011. High efficiency bioethanol production from OPEFB using pilot pretreatment reactor. *J. Chem. Technol. Biotechnol.* 86, 1527–1534.
- Hansen, R., 2013. Sweet Corn Profile. AgMRC, [http://www.agmrc.org/commodities\\_products/grains\\_oilseeds/corn\\_grain/sweet-corn-profile/](http://www.agmrc.org/commodities_products/grains_oilseeds/corn_grain/sweet-corn-profile/).
- Himmel, M.E., Ding, S.-Y., Johnson, D.K., Adney, W.S., Nimlos, M.R., Brady, J.W., Foust, T.D., 2007. Biomass recalcitrance. Engineering plants and enzymes for biofuels production. *Science* 315, 804–807.
- Jung, Y.H., Kim, I.J., Kim, J.J., Oh, K.K., Han, J.-I., Choi, I.-G., Kim, K.H., 2011. Ethanol production from oil palm trunks treated with aqueous ammonia and cellulase. *Bioresour. Technol.* 102, 7307–7312.
- Karunanithy, C., Muthukumarappan, K., 2013. Thermo-mechanical pre-treatment of feedstocks. In: Gu, T. (Ed.), *Green Biomass Pretreatment for Biofuels Production*, SpringerBriefs in Green Chemistry for Sustainability, pp. 31–65. [http://dx.doi.org/10.1007/978-94-007-6052-3\\_2](http://dx.doi.org/10.1007/978-94-007-6052-3_2).
- Kristiani, A., Effendi, N., Aristiawan, Y., Aulia, F., Sudiyani, Y., 2015. Effect of combining chemical and irradiation pretreatment process to characteristic of oil palm's empty fruit bunches as raw material for second generation bioethanol. *Energy Procedia* 68, 195–204.
- Lau, M.J., Lau, M.W., Gunawan, C., Dale, B.E., 2010. Ammonia fiber expansion (AFEX) pretreatment, enzymatic hydrolysis, and fermentation on empty palm fruit bunch fiber (EPFBF) for cellulosic ethanol production. *Appl. Biochem. Biotechnol.* 162, 1847–1857.
- Lima, M.A., Lavorente, G.B., da Silva, H.K.P., Bragatto, J., Rezende, C.A., Bernardinelli, O.D., deAzevedo, E.R., Gomez, L.D., McQueen-Mason, S.J., Labate, C.A., Polikarpov, I., 2013. Effects of pretreatment on morphology, chemical composition and enzymatic digestibility of eucalyptus bark: a potentially valuable source of fermentable sugars for biofuel production – part 1. *Biotechnol. Biofuels* 6, 1–17.
- Maryana, R., Märifatun, D., Wheni, A.I., Satriyo, K.W., Angga Rizal, W., 2014. Alkaline pretreatment on sugarcane bagasse for bioethanol production. *Energy Procedia* 47, 250–254.

- Millati, R., Wikandari, R., Trihandayani, E.T., Cahyanto, M.N., Taherzadeh, M.J., Niklasson, C., 2011. Ethanol from oil palm empty fruit bunch via dilute-acid hydrolysis and fermentation by *Mucor indicus* and *Saccharomyces cerevisiae*. *Agric. J.* 6, 54–59.
- Millati, R., Mustikaningrum, G., Yuliana, A., Cahyanto, M.N., Niklasson, C., Taherzadeh, M.J., 2014. Thailand 2nd generation ethanol by zygomycetes fungi at elevated. *Energy Procedia* 52, 104–109.
- Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31, 426–428.
- Mood, S., Golfeshan, A.H., Tabatabaei, M., Jouzani, S.G., Najafi, G., Gholami, M., Ardjmand, M., 2013. Lignocellulosic biomass to bioethanol; a comprehensive review on pretreatment. *Renew. Sust. Energ. Rev.* 27, 77–93.
- Nguyen, Q.A., 2014. Process for producing ethanol from agave bagasse and agave leaves. Patent WO2014209752 A1.
- Pan, X., 2008. Role of functional groups in lignin inhibition of enzymatic hydrolysis of cellulose to glucose. *J. Biobased Mater. Bio.* 2, 25–32.
- Perez-Pimienta, J.A., Lopez-Ortega, M.G., Chavez-Carvayar, J.A., Varanasi, P., Stavila, V., Cheng, G., Singh, S., Simmons, B.A., 2015. Characterization of agave bagasse as a function of ionic liquid pretreatment. *Biomass Bioenergy* 75, 180–188.
- Ragauskas, A.J., Williams, C.K., Davison, B.H., Britovsek, G., Cairney, J., Eckert, C.A., Frederick, W.J., Hallett, J.P., Leak, D.J., Liotta, C.L., Mielenz, J.R., Murphy, R., Templer, R., Tschaplinski, T., 2006. The path forward for biofuels and biomaterials. *Science* 311, 484–489.
- Ravindran, R., Jaiswal, A.K., 2015. A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: challenges and opportunities. *Bioresour. Technol.* 199, 92–102.
- Rocha, G.J.M., Gonçalves, A.R., Oliveira, B.R., Olivares, E.G., Rossell, C.E.V., 2012. Steam explosion pretreatment reproduction and alkaline delignification reactions performed on a pilot scale with sugarcane bagasse for bioethanol production. *Ind. Crops Prod.* 35, 274–279.
- Román, A., Garrote, G., Ballesteros, I., Ballesteros, M., 2013. Second generation bioethanol from steam exploded *Eucalyptus globulus* wood. *Fuel* 111, 66–74.
- Sambusiti, C., Licari, A., Solhy, A., Aboukhas, A., Cacciaguerra, T., Barakat, A., 2015. One-pot dry chemo-mechanical deconstruction for bioethanol production from sugarcane bagasse. *Bioresour. Technol.* 181, 200–206.
- Singh, R., Shukla, A., Tiwari, S., Srivastava, M., 2014. A review on delignification of lignocellulosic biomass for enhancement of ethanol production potential. *Renew. Sust. Energ. Rev.* 32, 713–728.
- Van Soest, P.J., Wine, R.H., 1968. Determination of lignin and cellulose in acid detergent fiber with permanganate. *J. AOAC Int.* 51, 780–784.
- Sudiyani, Y., Styarini, D., Triwahyuni, E., Sudiarmanto, Sembiring, K.C., Aristiawan, Y., Abimanyu, H., Han, M.H., 2013. Utilization of biomass waste empty fruit bunch fiber of palm oil for bioethanol production using pilot-scale unit. *Energy Procedia* 32, 31–38.
- Vandenbossche, V., Doumeng, C., Rigal, L., 2014a. Thermomechanical and thermo-mechano-chemical pretreatment of wheat straw using a twin-screw extruder. *BioResources* 9, 1519–1538.
- Vandenbossche, V., Brault, J., Vilarem, G., Hernández-Meléndez, O., Vivaldo-Lima, E., Hernández-Luna, M., Barzana, E., Duque, A., Manzanares, P., Ballesteros, M., Mata, J., Castellón, E., Rigal, L., 2014b. A new lignocellulosic biomass deconstruction process combining thermo-mechano chemical action and bio-catalytic enzymatic hydrolysis in a twin-screw extruder. *Ind. Crops Prod.* 55, 258–266.
- Vandenbossche, V., Brault, J., Vilarem, G., Rigal, L., 2015. Bio-catalytic action of twin-screw extruder enzymatic hydrolysis on the deconstruction of annual plant material: case of sweet corn co-products. *Ind. Crops Prod.* 67, 239–248.
- Velazquez-Martí, B., Fernandez-Gonzalez, E., Lopez-Cortes, I., Salazar-Hernandez, D. M., 2011. Quantification of the residual biomass obtained from pruning of vineyards in Mediterranean area. *Biomass Bioenergy* 35, 3453–3464.
- Xu, J.K., Chen, J.H., Sun, R.C., 2015. Hydrothermal microwave valorization of eucalyptus using acidic ionic liquid as catalyst toward a green biorefinery scenario. *Bioresour. Technol.* 193, 119–127.
- Zheng, J., Rehmann, L., 2014. Extrusion pretreatment of lignocellulosic biomass: a review. *Int. J. Mol. Sci.* 15, 18967–18984. <http://dx.doi.org/10.3390/ijms151018967>.
- Zhu, J.Y., Pan, X.J., 2010. Woody biomass pretreatment for cellulosic ethanol production: technology and energy consumption evaluation. *Bioresour. Technol.* 101, 4992–5002.
- Zhu, Z., Zhu, M., Wu, Z., 2012. Pretreatment of sugarcane bagasse with NH<sub>4</sub>OH-H<sub>2</sub>O<sub>2</sub> and ionic liquid for efficient hydrolysis and bioethanol production. *Bioresour. Technol.* 119, 199–207.