Biomass pretreatment with reactive extrusion using enzymes: A review

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ABSTRACT

Introducing enzymes during the extrusion process has been mainly used as new pretreatment techniques in the starch degradation process and, more recently, in the second generation bioethanol production. The technique, called the bioextrusion, is a special case of reactive extrusion. Starch and lignocellulose bioextrusion examples underline the good mixing capacities as a way to initiate the enzymatic reaction in high solid content conditions. Starch bioextrusion results show a low dextrinization yield but a real effect on the polymer size decrease which allows higher and faster subsequent saccharification. It also considerably reduced the recrystallization phenomenon that limits the saccharification efficiency. Bioextrusion of lignocellulose resulted in a better sugar production. Very short residence times limit the use of bioextrusion to a pretreatment technique. However, unique flexibility of the extrusion technique allows to subsequently pretreat, in the same extruder, with physical and/or chemical constraining conditions, followed by a milder bioextrusion.

1. Introduction

Bioextrusion is defined as the use of one or several types of enzymes as biocatalysts during the extrusion process. The extruders' adaptability allows the introduction of liquid enzyme solutions at different steps of the process. Even if all the known examples of this combination concern the treatment of biomasses, theoretically, all kinds of substrates can be processed with bioextrusion. Enzyme deactivation by extrusion has also been studied on different food products like cereal grains (Fretzdorff and Seiler, 1987) and fish muscles (Choudhury and Gogoi, 1996). These studies intended to deactivate enzymes involved in the product degradation in order to improve product shelf life (Linko et al., 1981). Proteins involved in the product degradation like proteases, lipases, lipoxidases, ureases and peroxidases, can be denatured thanks to shear fields and high temperatures. In these cases enzymes are not used as biocatalysts and do not correspond to the bioextrusion definition. This is why it won't be detailed in this review. This review summarizes the advantages and limitations of the combination between the extrusion process and the enzyme biotechnology. These characteristics will be studied through two main examples of its application, starch liquefaction and lignocellulose deconstruction.

1.1. A specific case of reactive extrusion

The extrusion is a continuous process that can easily be brought to an industrial level. This characteristic has led the early developments of this technique. It is a determining factor regarding volumes and quantities of biomasses that are processed for the food, feed or chemicals production. It is a flexible and versatile technique. Screw extruders are characterized by a highly versatile screw configuration which allows various constraint profiles. It can handle different substrates with different viscosities, rheological behaviors, phases. Processing conditions can easily be adapted and extrusion modules can be changed to add or remove functionalities (physical separation, degassing) (Bouvier and Campanella, 2014).

As enzymes can be seen as biocatalysts, bioextrusion can be regarded as a specific case of reactive extrusion. Historically chemical and biochemical reactions have been carried out in diluted conditions. But these conditions imply the use of solvents or diluents from 5 to 20 times the weight of the desired product, requiring costly facilities to recycle it. As shown by Vergnes and Berzin (2004), contrary to batch reactors, extruders can handle very viscous materials. Higher physical and chemical modification rates place it as an intensification process. In addition, low processing volumes and residence times permit to make economies in energy, materials and consumables. With increasing concerns about energy and environmental issues, efficient and continuous reactors like extruders are seen as sustainable processing technologies (Janssen, 2004). While solving the problem regarding the solvents use, it raises issues linked to the mixing of high viscous reactants or products and difficulties to achieve homogeneous mixtures.

Abbreviations: BAB, blue agave bagasse; BS, barley straw; DE, dextrose equivalent; GPC, gel permeation chromatography; HTST, high temperature and short time extrusion; OPEFB, oil palm empty fruit bunch; SC, sweet corn co-products; SEM, scanning electron microscopy; SME, specific mechanical energy; WSI, water solubility index

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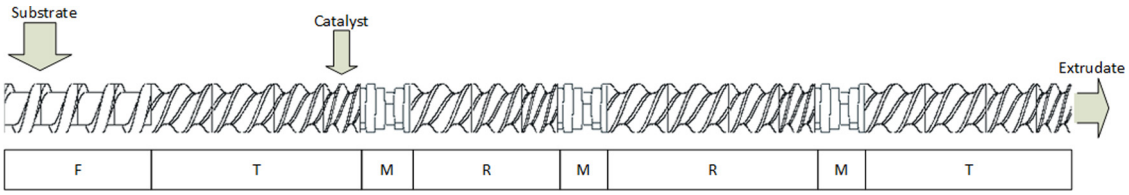

Fig. 1. reactive extrusion standard screw configuration. F corresponds to a feeding zone, T and R are respectively transport and reaction areas with conveying elements and M are mixing zones with kneading discs.

Extruders are continuous reactors and in line addition of reactants associated with restrictive screw elements (reverse or kneading blocks) permit to isolate specific barrel modules in order to carry out discrete chemical processes (Bouvier and Campanella, 2014). An example of this screw configuration is presented in Fig. 1. Four main classes of chemical reactions are realized in screw extruders, bulk reactions, control of molecular weight of polymers, chemical modifications and reactive li quid/solid extraction pressing. These reactions can be applied to syn thetic materials as well as biomaterials such as biomass. Reactive ex trusion has especially been studied in continuous monomers polymerization in bulk reactions, avoiding the use of solvents while enhancing process productivity and flexibility (Brown, 1991). It is still highly complicated to build a model of these reactions due to the lack of relevant kinetic data that were, for most of it, obtained in diluted conditions. Most of these reactions are carried out in intermeshing co rotating twin screw extruders.

Even though this technique provides many advantages, two main drawbacks are to be highlighted. Firstly the temperature regulation difficulties, because of the limited cooling capacities. Secondly, and most importantly, the limited residence time. In general it is comprised between 1 5 min, allowing only fast reactions to occur. In these fast conditions, species distribution depend on both bulk flow and mole cular diffusion (Bouvier and Campanella, 2014).

1.2. Enzymatic hydrolysis of biomass

Processing biomass brings issues that have already been solved by some living organisms. Enzymes are the key tools selected by the evo lution to efficiently degrade and assimilate biomass, from storage or gans (seeds, tubers, rhizomes) to structural organs with complex lig nocellulosic composition. Historically enzymes have served as efficient biocatalysts for biomass. Processes involving fermentation such as brewing, baking and the production of alcohol have been known and used as controlled natural transformations since prehistoric times. From its discovery in 1887 by Kühne to its wide application today, enzyme catalysis has progressively been developed in the biomass processing, from food to non food sectors.

In the 1960s the first enzyme preparation (amyloglucosidase) was produced, allowing the complete breakdown of the starch polymer into glucose. Greater yields, higher degree of purity and facilitated crystal lization led to the rapid conversion of the glucose production from acid hydrolysis to enzymatic hydrolysis. Later, in the 1990s, enzyme tech nology was applied for the degradation of complex polysaccharides present in plant cell walls. This was done in order to improve the ex traction of intracellular components in fruits (production of fruit juices) and seeds, involving enzymes such as pectinases, hemicellulases, and cellulases. Firsts applications in the non food industry have been de veloped at the same time within the textile, paper and more recently biofuels industries (Rehm and Reed, 1998). Enzyme catalysis is as an alternative to conventional physical, physiochemical or chemical treatments. Because of the rapid development of biotechnologies it has been more accessible for large scale applications (Guzmán Maldonado et al., 1995; Norman, 1981). It offers different advantages compared with conventional treatments that can be underlined concerning the biomass hydrolysis. The better specificity limits the production of by products that can later be inhibitory for the process, while increasing the reaction yield. These reactions can be done under mild conditions, using non toxic solvents which is particularly important in the food industry where products have to be edible and respect norms of food safety (Rosenthal et al., 1996). However, some limitations concerning biomass processing have to be cited. The main limitation is the reaction time that can extend to several days. Moreover, as highlighted in the 1.1 section, an optimal catalysis reaction requires diluted reaction conditions. Therefore the solid to liquid ratio is an important criterion since the enzyme, in its free form, needs a vector to move from a substrate to another. Increasing the solid concentration decreases the enzyme's mobility. It is observed in solid/liquid mixtures of insoluble substrates like lignocellulose, as well as with soluble substrate in high concentration that give highly viscous solution with diffusion limitation (Baks et al., 2008). In the case of insoluble lignocellulosic substrate, hydrolysis is limited by several factors. Particles size, available surface area, crystallinity of the cellulose, moisture content, lignin content and polymerization degree control the biomass accessibility (Hendriks and Zeeman, 2009). The use of one or several pretreatment methods is often required to improve the enzyme accessibility and therefore catalysis.

1.3. Twin screw extrusion: thermomechanical pretreatment of biomass

Extrusion of biomass historically appeared in the food industry of the mid 1930s with the production of pasta and the cereal processing industry. Kneading, cooking, forming and texturizing functions of the extruder are used in the cereals transformation. Extrusion also widely developed in the oilseed processing industry because of the fractiona tion capacities of the machine (Harper, 1981). More recently, in the 1980s, extrusion process has been applied to lignocellulosic biomass degradation, for applications such as paper industry (De Choudens et al., 1989), components extraction (N'Diaye et al., 1996) or pre treatment for bioethanol production, as summarized by (Zheng and Rehmann, 2014). As detailed earlier, extrusion pretreatment presents unique advantages and flexibility. Biomass processing commonly uses the high shear, high pressure and temperature capabilities of the ex truder, to physically deconstruct feedstock. With chemical resistant materials, it is also used for acid or alkali reactive treatments. These hard conditions that are enabled during the extrusion process aren't compatible with enzyme mild conditions. This is why it won't be de tailed in this review, especially since Karunanithy and Muthukumarappan (2013) already summarized the thermomechanical pretreatment of a large range of feedstocks. Among other advantages, it points out the good mixing capacities that removes the softened parts while exposing the interior surface to thermal and chemical action, thus improving the material deconstruction. And finally, it has to be un derlined that the flexible and continuous aspects of the extruder allow to sequentially pretreat biomass while permitting, in a subsequent part of the machine, good conditions for enzyme catalysis, which is one of the main advantage of this technology (Vandenbossche et al., 2016).

2. Bioextrusion: combining thermomechanical treatment with enzyme catalysis

2.1. Bioextrusion of starch: starch liquefaction

As it was underlined in the 1.2 section, starch degradation is one of the first and main sector to use enzyme biotechnologies. The industrial process of starch hydrolysis permits the production of a variety of glucose and glucose based products, from glucose syrups (mixtures of polymers of D glucose), to dextrose hydrolysates with the maximum amount of low molecular weight sugars (Guzmán Maldonado et al., 1995). These hydrolysates are conventionally produced including two processing steps. First, the liquefaction step (30 90 min), includes the gelatinization, corresponding to the opening of the starch structure by hydration of the polymer to facilitate the enzyme attack, and the dex trinization, which prevents the retrogradation or crystallization during the subsequent steps. Secondly, the saccharification step (12 96 h), is the enzyme breakdown of the liquefied mixture of oligo and poly saccharides into low molecular weight sugars.

On the one hand High Temperature Short Time (HTST) extrusion has been widely used in the cereal grain processing industry (Linko et al., 1981). Mercier and Feillet (1975) showed that low moisture level starches can be liquefied by continuous extrusion cooking. Yet ther momechanical treatment alone is limiting for two main reasons. Firstly a rapid retrogradation phenomenon takes place when the extrudate cool below 60 °C (up to 18% of the dry weight). Secondly produced starches slurries are highly viscous thus leading to handling difficulties in the case of a following continuous saccharification process (Linko et al., 1983a). On the other hand the traditional acid hydrolysis of starch has been replaced by the enzyme conversion (Norman, 1981; Rehm and Reed, 1998). The old acid technique is non specific, gives a low product yield, leads to the formation of by products and requires the use of a base in order to neutralize the solution which implies the use of expensive corrosion resistance materials. As seen in the 1.2 section, enzyme catalysis is a solution to these issues but requires a long reaction time (24 96 h), a high enzyme concentration (up to 21 AGI/g, 2,34 U/g) and becomes difficult at high solid concentrations (handling and repolymerization issues at 30 40%) (Govindasamy, 1995; Labout, 1985).

Several publications focus on the introduction of enzymes during the extrusion process, reinforced by the observation that the α amylase could keep its activity during and after the extrusion process (Linko et al., 1978). Different sources of starch have been tested such as corn (*Zea mays* L.) (Chouvel et al., 1983), barley (*Hordeum vulgare*) (Linko et al., 1983a), wheat (*Triticum aestivum*) (Hakulin et al., 1983; Reinikainen et al., 1986), sago (*Metroxylon sagu*) (Govindasamy et al., 1997a) and more recently broken rice (*Oryza sativa*) starch (Li et al., 2013; Xu et al., 2017, 2015). Due to the time needed for the complete saccharification, the bioextrusion has mainly been limited to the li quefaction part, but some researchers intended to initiate the sacchar ification step as well (Hakulin et al., 1983; van Zuilichem et al., 1990). Thermostable α amylases from *Bacillus licheniformis* are introduced for the liquefaction step with an optimal temperature of 90 °C and an pH optimum between 6.0 6.5 (Ivanova et al., 1993). Saccharification step is realized with fungal glucoamylases with temperature optima between 50 and 70 °C and optimal pH observed from 4.5 to 6.5.

2.1.1. Factors influencing starch bioextrusion

Thanks to the numerous publications and the different response surface methodologies used, it is possible to summarize the main in fluencing factors. The degree of starch gelatinization, enzyme activity during extrusion and efficiency of the subsequent saccharification stage are mainly influenced by enzyme concentration, moisture content and temperature. Other less investigated yet influencing factors are re sidence time and screw speed and configuration. Bioextrusion tested conditions are summarized into Table 1a (extruders characteristics) and Table 1b (enzymes and substrates characteristics).

2.1.1.1. Enzyme loading. First of all, increasing the α amylases concentration increases the liquefaction efficiency. Most of the authors obtained the highest initial and subsequent hydrolysis rate with the highest concentration tested (Table 1b). Chouvel et al. (1983), showed a linear response between the extend of hydrolysis and the α amylases loading.

2.1.1.2. Enzyme combination. Some publications also combined the addition of α amylases with the addition of glucoamylases, thus starting the saccharification stage at the end of the extrusion process, Hakulin et al. (1983) observed a higher conversion level when introducing these glucoamylases into the last extrusion section, while van Zuilichem et al. (1990) measured a positive influence when increasing both α amylases and glucoamylases (Table 1b).

2.1.1.3. Moisture content. Similarly to enzyme concentration, all the publications regarding the bioextrusion of starch observed a better effect with higher moisture content. If extruders can work at low moisture content with high viscosity conditions, a higher feed moisture increases the bioextrusion effect by allowing an easier enzyme diffusion. Moreover, lowering the moisture content leads to higher mechanical forces that can denature enzymes (Baks et al., 2008; Kaya et al., 1994). Best operating moisture were often found at the high range of the one tested (Table 1b).

2.1.1.4. Temperature. The temperature is another influencing factor. Operational temperatures are limited by the thermo degradation of enzymes. Several researchers found optimal temperatures of the system in the 90 100 °C range (Tables 1a and 1b), reporting better results when α amylase activity was preserved. With a half life of 15 min at 110 °C, the short time residence limits the denaturation (van Zuilichem et al., 1990). However researchers that selected temperatures higher than 100 °C observed partial deactivation, up to 160 °C, where almost complete inactivation of the enzyme occurred (Linko et al., 1983b). In the case where saccharification was initiated in the extruder, saccharification zones where even set up lower (60 65 °C) (Hakulin et al., 1983).

2.1.1.5. Screw speed and configuration. The extrusion profiles are often corresponding to the classic organization seen in reactive extrusion. A repeated configuration of transport elements (forward screw elements) followed by an intensive mixing zone with kneading discs create isolated areas for reactions to occur (Fig. 1). Mechanical constraints are not a center of interest in the many studies concerning starch bioextrusion. It has yet been observed that the application of mechanical forces reduce the thermal energy needed to deconstruct and gelatinize the starch (Baks et al., 2008). Reinikainen et al. (1986), investigated the effect of restrictive screw elements. The increase of the counter screw length led to an increase of the degree of hydrolysis at the lower temperature range (close to 100 °C). The regulation of screw speed can also modify the mechanical force applied on the extrudate (Baks et al., 2008).

As underlined in the 2.1.1.3 section, physical constraints are highly dependent on the water content as observed by Lawton et al. (1972). A high water content will require higher temperatures or faster screw speed. In the bioextrusion case this factor is limited by the protein degradation, high temperatures and high mechanical frictions will limit the enzyme action.

Besides, Govindasamy et al. (1997a, 1997b) investigated both single and twin screw bioextrusion. They observed a preferential degradation of the amylopectin fraction for the single screw and no significant effect on the amylose fraction. Twin screw bioextrusion had visible impacts on the GPC chromatograms on both fractions. Most of the bioextrusion publications focus on the twin screw extrusion. Bouvier et Campanella

Table 1a
Summary of starch bioextrusion characteristics. extruders characteristics and corresponding responses (Hakulin et al., 1983; Linko et al., 1983b; Reinikainen et al., 1986; van Zuilichem et al., 1990; Govindasamy et al., 1997a,b; Vasanthan et al., 2001; Baks et al., 2008; de Mesa-Stonestreet et al., 2012; Li et al., 2013; Xu et al., 2017).

Publication	*Extruder*	*Length (L)*	*Diameter (D)*	*Length/Diameter (L/D)*	*Bioextrusion length*			*Screw speed*		*Barrel temperature*	
#	#	*mm*	*mm*		*mm*			*rpm*		*°C*	
								min	*max*	*min*	*max*
Hakulin et al.. 1983	*Werner & Pfleiderer Continua 58 twin-screw*	1160	58	20	L or around (1/3) L glucoamylases 145 mm			250		150	200
Linko et al.. 1983b	*Creusot-Loire BC-45 twin-screw*	600	55	120/11	L			150		n/a	
Reinikainen et al.. 1986	*Creusot-Loire BC 45 twin-screw*	600	55	120/11	L			150		n/a	
van Zuilichem et al.. 1990	*Cincinnati Milacron CM 45*	n/a	45	n/a	100 mm from the feed point glucoamylases just before the die			32		n/a	
Govindasamy et al.. 1997	*Clextral BC 21 twin-screw*	400	25	16	L	Conditions		70	190	70	130
						Results				105 *opt*	
						Time (h)	#			*min*	*max*
						0	OC			0	22
							DE			0	10 *opt*
							WSI			11	60 *opt*
Vasanthan et al.. 2001	*Werner and Pfleiderer ZSK 57W 50P. Stuttgart. Germany*	n/a	n/a	20	n/a	Conditions		50		90	140
						Results				100 *opt*	
						Time (h)	#			*min*	*max*
						0	DH[3]			0	27.8 *opt*
							DE[3]			1.6	36.1 *opt*
							DH[4]			0	21.8 *opt*
							DE[4]			1.1	31.3
Baks et al.. 2008	*Berstorff. Hannover. Germany*	1000	25	40	around L/4.5			50	150	60	90
						Conditions		100 *opt*		90 *opt*	
						Results					
						Time (h)	#	*Min to max*			
						0	DE	[1]0 to 9 *opt*			
						1	DE	[1]8 to 40 *opt*			
						24	DE	[1]32 to 57 *opt*			
de Mesa-Stonestreet et al.. 2012	*M-18. American Leistritz. Somerville.NJ*	522	18	29	L	Conditions		350		n/a	
Li et al.. 2012	*TSE 24 MC. Thermo scientific. USA*	n/a	n/a	40	L	Conditions		100		90	106
						Results				98 *opt*	
						Time (h)	#	*Min to max*			
						600	FE	[1]69.57 to 93.63 *opt*			
Xu et al.. 2017	*TYPE TSE 24 MC. Thermo scientific. USA*	600	24	25	L	Conditions		100	200	80	100
						Results		100 *opt*		100 *opt*	
						Time (h)	#	*Min to max*			
						0	SG	[1]63.5 to 99.9 *opt*			

Extruders characteristics and conditions are summarized in this table. A results section is presented under the bioextrusion conditions part of each publication. These sections gather the different results obtained. and its corresponding time. which describe the starch liquefaction. These are not exhaustive results but give an idea of the process impact and efficiency with ranges between minima and maxima obtained.
DE is the dextrose equivalent; DH is the degree of hydrolysis; OC is the oligosaccharides content; WSI is the water soluble index; FE is the fermentation efficiency; SG is the starch gelatinization.
[opt]Indicates the optimum values observed in the conditions and results section. In the results section. values are referenced as [opt]If it has been measured with the corresponding optima indicated in the conditions section.
[1]Centered values are obtained with multiple factor modifications (often thanks to a response surface model) that are not associable with a single specific condition.

2014b underline better mixing capacities and product uniformity with twin screw extrusion that could explain this choice.

2.1.1.6. Residence time. The residence time influence was not widely studied in the case of starch bioextrusion. Hakulin et al. (1983), tested the residence time influence by modifying the enzyme addition stage. These modifications have only a small impact on the residence time that can be measured in seconds, and the results showed really close values (DE around 90). Govindasamy (1995), observed dextrose equivalent (DE) and water solubility index (WSI) values slightly higher when enzymes were injected earlier in the extruder. Vasanthan et al. (2001) observed lower starch gelatinization when increasing the screw speed. They associated this result with the decrease of the residence time, knowing that it also modifies the mechanical forces applied.

Table 1b

Summary of starch bioextrusion characteristics with enzymes, substrates characteristics and corresponding responses (Hakulin et al., 1983; Linko et al., 1983b; Reinikainen et al., 1986; van Zuilichem et al., 1990; Govindasamy et al., 1997a,b; Vasanthan et al., 2001; Baks et al., 2008; de Mesa-Stonestreet et al., 2012; Li et al., 2013; Xu et al., 2017).

Publication			*Feed rate (kg/h)*		*Feed moisture (%)*		*Mass temperature (°C)*		*Enzyme concentration (%)*				*Enzyme*	*Biomass*
									α-amylase		*gluco-amylase*			
			min	*max*	*min*	*max*	*min*	*max*	*min*	*max*	*min*	*max*		
Hakulin et al., 1983	Conditions		0.9	1.5	40	65	90	127	0.9		0	0.66[1]	*Termamyl 120L Glucoamylase 150 L*	*Wheat starch*
	Results				65 [opt]						0.66 [opt]			
	Time (h)	#			*min*	*max*					*min*	*max*		
	0[2]	DE					[3]17.4 to 32.1							
	1	DE									63	75 [opt]		
	5	DE									86	94 [opt]		
	10	DE			85	92 [opt]								
	22	DE					[3]86.8 to 97.2 [opt]							
Linko et al., 1983b	Conditions		12		40	60	105	160	0	3	0		*Termamyl 60L Thermamyl 120L*	*Native barley starch*
	Results				60 [opt]		125 [opt]		3 [opt]					
	Time (h)	#			*min*	*max*	*min*	*max*	*min*	*max*				
	0	IM			1.8 [opt]	2.9			5.5 [opt]	18.5				
	1/6	DE			16	24 [opt]	2	24-27						
	10	DE					76	90-95 [opt]	75-80	88-95 [opt]				
	24	DE			93	96 [opt]								
	48	DE							85-88	95 [opt]				
Reinikainen et al., 1986	Conditions		12		35	65	100	150	0	6 (ml/kg)	0		*Termamyl 60L*	*Wheat starch*
	Results													
	Time (h)	#					*min to max*							
	0	DH					[3]<1							
		DE					[3]0.27 to 2.7							
	2	DE					[3]1.2 to 23							
van Zuilichem et al., 1990	Conditions		11		60	80	120		0	0.262	0.05	1	*Termamyl 120L Amigase GM*	*Cracked corn*
	Results													
	Time (h)	#					*min to max*							
		1/A					[3]0 to >400							
		1/B					[3]<55 to >75							
Govindasamy et al., 1997	Conditions		2		28.5	50.5	n/a		0	1	0		*Termamyl 120L*	*Sago starch*
	Results				50.5 [opt]				1 [opt]					
	Time (h)	#			*min*	*max*			*min*	*max*				
	0	DH					[3]0.38 to 1.82 [opt]							
		DE							0	10 [opt]				
		OC			0	22 [opt]								
		WSI					[3]0 to 60 [opt]							
Vasanthan et al., 2001	Conditions		22.7		20	50	n/a		0	4	0		*Termamyl 120L*	*Barley grains*
	Results				50 [opt]				4 [opt]					
	Time (h)	#					*min to max*							
	0	DH[3]					[3]0 to 27.8 [opt]							
		DE[3]					[3]1.6 to 36.1 [opt]							
		DH[4]					[3]0 to 21.8 [opt]							
		DE[4]					[3]1.1 to 31.3 [opt]							
Baks et al., 2008	Conditions		2		30	50	n/a		0.1	2.5	0		*Termamyl 120 L*	*Native wheat starch*
	Results				40 [opt]				2.5 [opt]					
	Time (h)	#					*min to max*							
	0	DE					[3]0 to 9 [opt]							
	1	DE					[3]8 to 40 [opt]							
	24	DE					[3]32 to 57 [opt]							
de Mesa-Stonestreet et al., 2012	Conditions		1.48	2.25	14	50	n/a		0	10	0		*Liquozyme SC DS*	*Sorghum flour*
Li et al., 2012			1.5		30	42	n/a		0.01	0.13	0			
Xu et al., 2017	Conditions												*Termamyl 120 L*	*Milled broken rice*
	Results				42 [opt]				0.1 [opt]					
	Time (h)	#					*min to max*							
	600	FE					[3]69.57 to 93.63 [opt]							
	Conditions		1.5	3	22	38	n/a		0	0.1	0		*Termamyl 120 L*	*Glutinous rice*
	Results		3 [opt]		38 [opt]				0.1 [opt]					
	Time (h)	#					*min to max*							
	0	SG					[3]63.5 to 99.9 [opt]							

Substrates and enzymes characteristics and conditions are summarized in this table. A results section is presented under the bioextrusion conditions part of each publication. These sections gather the different results obtained, and corresponding time, which describe the starch liquefaction. These are not exhaustive results but give an idea of the process impact and efficiency with ranges between minima and maxima obtained. The 0 value in the time column of the result section corresponds to the end of the extrusion process. Unless indicated otherwise, enzyme concentrations are expressed as the ratio of protein (g) per dry biomass (g).
DE is the dextrose equivalent; IM is the quantity of insoluble material; DH is the degree of hydrolysis; 1/A is the initial conversion rate; 1/B is the final conversion level; OC is the oligosaccharides content; WSI is the water soluble index; FE is the fermentation efficiency; SG is the starch gelatinization.
[1]Last segment was cooled down to 60 °C and feed moisture was adjusted to 70% when gluco-amylases where added.
[2]Indicated as the beginning of the batch process, without any precision on the time needed to transfer the bioextrudate into the batch.
[3]Centered values are obtained with multiple factor modifications (often thanks to a response surface model) that are not associable with a single specific condition.
[opt]Indicates the optimum values observed in the conditions and results sections. In the results section, values are referenced as [opt] if it has been measured with the corresponding optima indicated in the conditions section.

2.1.2. Bioextrusion of starch results

2.1.2.1. Prevent the retrogradation phenomenon. Some common clear advantages can be drawn out of the different results obtained. The main advantage of introducing enzymes is to minimize or even eliminate the retrogradation of the liquefied starch. This phenomenon can lead to a recrystallization as high as 18% of the dry weight (Table 1b). With the same DE observed in the soluble portion, a significant difference in the conversion rate after the saccharification step shows that the insoluble formation is a limiting factor affecting starch hydrolysis (Hakulin et al., 1983). α amylases were repeatedly proved to prevent this recrystallization during extrusion liquefaction by most of the authors (Reinikainen et al., 1986; van Zuilichem et al., 1990). Furthermore, Hakulin et al. (1983) observed a reduction of insolubles when saccharification was initiated during the extrusion, as a continuous process (Table 1b). The introduction of glucoamylases reduced the insoluble from 2 to 5% to 0.5% of the dry mass.

2.1.2.2. Reduction of cold paste viscosity. Another improvement observed with the bioextrusion technique is the reduction in product cold paste viscosity. High viscosity pastes are difficult to handle but are desirable in order to obtain a commercially feasible process and to reduce the water consumption. Specific Mechanical Energy (SME) is the conventional parameter used to measure the substrate viscosity. Govindasamy et al. (1997a) showed that SME decreased from 57 to 131 Wh/kg in the case of conventional extrusion processing to 21 91 Wh/kg when enzymes were added.

2.1.2.3. A faster and higher subsequent saccharification or fermentation. A DE of 89 was measured after 10 h for the bioextrudated sample, whereas it took 48 h to obtain a DE of 94 for samples gelatinized and liquefied in batch conditions (Linko et al., 1983a). Li et al. (2013) measured a 5% increase of alcoholic degree obtained after 5 days of fermentation for the bioextrudated broken rice and a final fermentation efficiency around 20% higher (Table 1b). These publications show slightly higher final DE and fermentation values with bioextrusion and moreover, DE values are obtained faster with the bioextrusion step (Table 1b).

2.1.3. Bioextrusion of starch effects comprehension

The bioextrusion process, with its short residence time results in an absence or a limited hydrolysis of starch. Govindasamy et al. (1997a) measured the DE and found values lower than 10 while Reinikainen et al. (1986) measured DE of less than 1 after extrusion (Table 1b). However, if hydrolysis is limited, SEC measurements showed a clear reduction of the molecular size during the process. MW lower than native starch (2×10^6) were produced during the bioextrusion pre treatment. A variety of smaller molecules increased the ratio MW < 2 $\times 10^6$/MW > 2 $\times$ l0^6 with the formation of a MW < 2000 fraction. Scanning electron microscopy of the starch granules after pretreatments gives interesting information about the qualitative action of bioextru sion over starch granules. When incubated with enzymes but without thermomechanical treatment, the external structure appears mainly intact and some internal pores are visible. On the other hand, with thermomechanical treatment and enzymes, below 100 °C, if the integrity of the granules is maintained, some breakings and more pores are observed. When in these conditions, with a temperature higher than 100 °C, but lower than the inactivation of enzymes, a complete loss of granular integrity is observed and a honeycomb structure appears which probably facilitates the enzyme penetration (Govindasamy et al., 1997b). Several authors also measured the WSI as an interesting in dicator of the material deconstruction. Xu et al., 2015 observed high WSI values with bioextrusion, 1.7 1.8 times higher than with other pretreatment methods. Together with low viscosity, low water ab sorption index, and visible physical denaturation, it confirms the pre vious observations. Bioextrusion isn't long enough to permit the sac charification but synergy between good mixing, shearing action of the extruder and initiation of the enzymatic hydrolysis, it leads to an effi cient material deconstruction, pretreatment method with high solid concentrations.

2.2. Bioextrusion of lignocellulose: lignocellulose deconstruction

Lignocellulose treatment using continuous twin screw extrusion started as an idea that emerged during a partnership between Clextral and the Technical Center for Paper in France in the late 1970s. It led to the development of extrusion pulping of non wood fibers for the paper production during the 1980s (De Choudens et al., 1989, 1984; Kurdin and Bohn, 1984). The introduction of enzymes during the extrusion process however started much more recently with the development of second generation bioethanol production. Being a cheap substrate, available in large quantity and everywhere on earth, lignocellulosic biomass appears to be a good alternative to fossil resources in deple tion. Its use, yet, remains limited by its complex structure and recalci trance to enzyme accessibility (Himmel et al., 2007).

2.2.1. Lignocellulosic biomasses and working conditions

Contrary to the bioextrusion of starch, bioextrusion of lig nocellulose, which is quite recent, hasn't been studied in details yet. However some experiments show interesting results regarding the material deconstruction and the production of fermentable sugars. Vandenbossche et al. (2014) tested several lignocellulosic substrates such as sweet corn co products (SC), blue agave (*Agave tequilana*) ba gasse (BAB), oil palm (*Elaeis guineensis Jasq.*) empty fruit bunch (OPEFB) and barley straw (BS). (Duque et al., 2014) worked with barley straw as well and (Samaniuk et al., 2011) with ensiled corn stover (Table 2a).

Even if it is complicated to make the comparison between all these studies, some common working conditions can be underlined. First of all the temperatures of bioextrusions where set around 50 °C (Table 2b), which is the optimal temperature for most of the cellulases cocktails, and mass temperatures ranged from 33 to 55 °C. All the publications cited in this part use pretreated lignocellulosic biomasses as starting material for the bioextrusion experiments. All biomasses where pre viously milled with particles size equal or smaller than 6 mm (Table 2c). Some authors had recourse to an alkali pretreatment (Duque et al., 2014; Vandenbossche et al., 2015, 2014), others used acid pretreated biomass (Samaniuk et al., 2011). All bioextrusion profiles are also si milar with several sequences of consecutive transport and mixing effect

Table 2a
Pretreated lignocellulosic biomasses compositions and bioextrudated biomasses compositions.

Publication	Biomass	Pretreated[a]			Bioextrudated[b]		
		Cellulose (% dwb)	Hemicellulose (% dwb)	Lignin (% dwb)	Cellulose (% dwb)	Hemicellulose (% dwb)	Lignin (% dwb)
Duque et al., 2014	Barley Straw	44.6	28.2	17.2	n/a	n/a	n/a
Vandenbossche et al., 2014	Sweet corn co-products	46.4	28.8	4.8	38.5	16.7	4.2
	Barley straw	49.5	18.8	11.8	36.6	6.8	9.4
	Blue agave bagasse	47.2	18.5	16.4	42.1	15.7	15.9
	Oil palm empty fruit bunch	53.1	23	21.4	37.9	14	15.6
Vandenbossche et al., 2015	Sweet corn co-products	46.4	28.8	4.8	n/a	n/a	n/a
Vandenbossche et al., 2016	Sweet corn	33	26	3	29	15	2
	Sugarcane bagasse	48	22	11	39	10	10
	Eucalyptus	52	9	20	36	8	19
	Vineyard pruning	37	12	15	32	10	9
	Blue agave bagasse	44	12	8	32	10	7
	Oil palm empty fruit bunch	38	16	10	38	14	9
Samaniuk et al., 2011	Corn stover	50	n/a	n/a	n/a	n/a	n/a

[a] Pretreated: extruded without adding enzymatic solution.
[b] Bioextruded: extruded after adding enzymatic solution.

Table 2b
Summary of lignocellulose bioextrusions characteristics, extruders characteristics.

Publication	Biomass	Extruder	Length (L)	Diameter (D)	Length/Diameter (L/D)	Screw speed		Barrel temperature(s)
#	#	#	mm	mm	%	rpm		°C
						min	max	
Duque et al., 2014	Barley Straw	Clextral EV 25	600	25	24	122		50
Vandenbossche et al., 2014	Sweet corn co-products	Clextral BC 21	700	24	29.17	250		50
	Blue agave bagasse	Clextral EV 25	1000	25	40	150		50
	Oil palm empty fruit bunch	Clextral EV 25	1000	25	40	85		50
	Barley straw	Clextral BC 21	700	24	29.17	200		40
Vandenbossche et al., 2015	Sweet corn co-products	Clextral BC 21	700	24	29.17	200		50
Vandenbossche et al., 2016[a]	Sweet corn	Clextral EV 53	1913.4	52.45	36.48	200		55/55/41/40/37
	Sugarcane bagasse	Clextral EV 53	1913.4	52.45	36.48	200		55/69/34/39/26
	Eucalyptus	Clextral EV 53	1913.4	52.45	36.48	200		55/75/49/36/27
	Vineyard pruning	Clextral EV 53	1913.4	52.45	36.48	200		58/60/25/25/24
	Blue agave bagasse	Clextral EV 53	1913.4	52.45	36.48	100		55/55/41/40/37
	Oil palm empty fruit bunch	Clextral EV 53	1913.4	52.45	36.48	175		58/78/35/35/35
Samaniuk et al., 2011	Corn stover	Modified torque rheometer	n/a	n/a	n/a	25	110	n/a

[a] Barrel temperatures listed by Vandenbossche et al., 2016 are real measured barrel temperatures of each module involved in the bioextrusion step.

using as presented in Fig. 1. Even with large extruder diameters, feed rates of lignocellulosic biomasses were kept relatively low (maximum 3.29 kg/h), whereas starch feed rates were set up to 22.7 kg/h. This is probably linked to the heterogeneity and handling difficulties of lig nocellulosic substrate, contrary to substrates as homogeneous and thin than flour. In all the lignocellulose bioextrusion publication extrudate moisture is higher than 70% (Table 2c). This is higher than most of the feed moisture used with starch (Table 1b). Again here it is probably linked to handling issues. Most of the lignocellulosic biomasses contains fibers than absorb a lot of water and are difficult to process without proper moisture. Finally, this is often the same enzymatic cocktail used with low enzyme concentrations in order to keep an economically vi able process. However, higher enzyme concentrations were used with the most resisting biomass in order to improve the sugar production (Oil palm empty fruit bunch, Tables 2a and 2c).

2.2.2. Lignocellulosic bioextrudates sugar composition and further fermentation

When measuring the amount of sugars released right after the bioextrusion, only small conversion rates were observed by the authors. Vandenbossche et al. (2015) observed a total sugar conversion/dry mass of 9% for the SC, which is nevertheless 5 times higher than without enzymes. Around 4% of glucose conversion/dry mass where measured by Samaniuk et al. (2011) after 5 min of bioextrusion, which is barely 1% more than without good mixing conditions. Vandenbossche et al. (2014) and Duque et al. (2014) incubated the bioextrudate into batch conditions and observed good polysaccharides conversion. More than 55% monomeric sugars/dry mass for the SC, BAB and BS and 26% for the OPEFB after 48 h and 70% yield of glucose and xylose after 72 h for the second. Table 2c summarizes glucose and xylose production at the end of the enzymatic hydrolysis in batch that follows the bioextrusion process. Vandenbossche et al. (2016) observed reduced hydrolysability for biomasses containing more lignin (Tables 2a and 2c). However, if there is probably a link, they underline that lignin is not the only influencing parameter.

2.2.3. Lignocellulosic biomass deconstruction

Even though these low sugar yields, biomass deconstruction is al ready observable. Duque et al. (2014) indicate a modification in the particle size distribution with 70% of the particles smaller than 1 mm,

Table 2c
Summary of lignocellulose bioextrusion characteristics with enzymes, substrates characteristics and corresponding responses (Duque et al., 2014; Vandenbossche et al., 2014; Vandenbossche et al., 2015; Vandenbossche et al., 2016; Samaniuk et al., 2011).

Publication		*Mass temperature*	*Liquid/Solid ratio*	*Feed rate*	*Enzyme concentration*	*Milled substrate size*	*Enzyme*	*Biomass*
#		*°C*	*%*	*kg/h*	*%*	*mm*	#	#
Duques et al., 2014	Conditions	n/a	3.3	0.62	2.5	5	*Novozyme Corp. Cellulases and Hemicellulases (9:1)*	*Barley Straw*
	Results							
	Time (h) #							
	72 *G (% of DM)*			32				
	72 *X (% of DM)*			18				
Vandenbossche et al., 2014b	Conditions	n/a	3.1	1.3	2.5	6	*Novozyme Corp. Cellulases and Hemicellulases cocktails*	*Sweet corn co-products*
	Results							
	Time (h) #							
	48 *G (% of DM)*			38				
	48 *X (% of DM)*			19				
	Conditions	n/a	3.1	0.5-1	2.5	5	*Novozyme Corp. Cellulases and Hemicellulases cocktails*	*Barley straw*
	Results							
	Time (h) #							
	48 *G (% of DM)*			38				
	48 *X (% of DM)*			17				
	Conditions	n/a	4	0.5	2	2	*Novozyme Corp. Cellulases and Hemicellulases cocktails*	*Blue agave bagasse*
	Results							
	Time (h) #							
	48 *G (% of DM)*			49.5				
	48 *X (% of DM)*			19.4				
	Conditions	n/a	4.35	1.46	11	2	*Novozyme Corp. Cellulases and Hemicellulases cocktails*	*Oil palm empty fruit bunch*
	Results							
	Time (h) #							
	48 *G (% of DM)*			16.4				
	48 *X (% of DM)*			9.6				
Vandenbossche et al., 2015	Conditions	n/a	2.5	3.29	4.6	6	*Advanced Enzyme Technologies (India) Cellulases and Hemicellulases cocktails*	*Sweet corn co-products*
	Results							
	Time (h) #			*min to max*				
	0 *TS (% of DM)*			<2[1] to 9				
	1,17 *TS (% of DM)*			<2[1] to 12				
	1,17 *FPC (%/DM)*			13[1] to 24				
Vandenbossche et al., 2016	Conditions	42	3.5	0.16	1.5	6	*Novozyme Corp. Cellulases and Hemicellulases (9:1)*	*Sweet corn co-products*
	Results							
	Time (h) #							
	24 *G (% of DM)*			26				
	Conditions	40	3.4	0.1	2.5	6	*Novozyme Corp. Cellulases and Hemicellulases (9:1)*	*Sugarcane bagasse*
	Results							
	Time (h) #							
	24 *G (% of DM)*			25				
	Conditions	45	3.4	0.1	2.5	6	*Novozyme Corp. Cellulases and Hemicellulases (9:1)*	*Eucalyptus*
	Results							
	Time (h) #							
	24 *G (% of DM)*			15				
	Conditions	33	3.4	0.1	2.5	6	*Novozyme Corp. Cellulases and Hemicellulases (9:1)*	*Vineyard pruning*
	Results							
	Time (h) #							
	24 *G (% of DM)*			20				
	Conditions	43	3.8	0.2	2.6	2	*Novozyme Corp. Cellulases and Hemicellulases (9:1)*	*Blue agave bagasse*
	Results							
	Time (h) #							
	24 *G (% of DM)*			33				
	Conditions	47	5.5	0.11	5.3	2	*Novozyme Corp. Cellulases and Hemicellulases (9:1)*	*OPEFB*
	Results							
	Time (h) #							
	24 *G (% of DM)*			18				
Samaniuk et al., 2011	Conditions	55	4	n/a	10FPU/g cellulose	3	*Novozyme Corp. Celluclast and cellobiases from Trichoderma reesei and Aspergillus niger (4:1)*	*Corn stover*
	Results							
	Time (h) #			*min to max*				
	0,5 *GC*[2]			7[4] to 10				
	0,5 *GC*[3]			5[4] to 6.5				

A results section is presented under the bioextrusion conditions part of each publication. These sections gather the different results obtained, and corresponding time, which describe the starch liquefaction. These are not comprehensive results but give an idea of the process impact and efficiency with ranges between minima and maxima obtained. In the results section, highest values are shown in bold if they coincide with the corresponding bold optimum process conditions. Unless indicated otherwise, enzyme concentrations are expressed as the ratio of protein (g) per dry biomass (g). G corresponds to glucose; X corresponds to xylose; TS corresponds to total sugars; FPC corresponds to fine particle content; GC corresponds to glucose conversion.
[1]Values obtained without enzymes.
[2]Values obtained with a mixing speed of 25 rpm
[3]Values obtained with a mixing speed of 55 rpm
[4]Values obtained without mixing.

whereas 70% of the particles are bigger than 1 mm inside the starting BS material. Vandenbossche et al. (2015) observed a physical de gradation of the bioextrudate fiber by SEM. They also obtained twice the ratio of hot water soluble compounds/dry mass when using en zymes, going from 20 to 40%, and a slight decrease in the dynamic viscosity for the SC. As shown by Table 2a, biomasses measured com position evolves during bioextrusion. There is a clear cellulose and hemicellulose decrease that would require more data to be associated with biomass composition or process conditions.

2.2.4. Bioextrusion of lignocellulose: residence time as a major influencing factor

As underlined in the 1.1 section, the main limitation of the reactive extrusion technique is the short residence time, with an estimated duration of a few minutes maximum. It is very short when compared with conventional reaction times of several days (3 7 days according to (Montague et al., 2002)) to obtain the maximum yield using the batch conditions. These conditions don't allow complete saccharification of the biomass. Vandenbossche et al. (2014), calculated a residence time between 1.5 3 min, in a latter publication they intended to overcome this limitation by recirculating the sweet corn co product (SC) bioex trudate up to 7 times corresponding to a total of 70 min including the time in between two consecutive runs (Vandenbossche et al., 2015). Samaniuk et al. (2011), used a torque rheometer in order to create a batch reactor with intensive mixing properties close to those existing within the extruder. Reactions were conducted during 40 min from when torque response was no longer a function of time. Influence of the screw speed can be noted as a small decrease in glucose conversion is visible when speed is increased from 25 to 55 rpm (Table 2c). In both cases, the bioextrudate became more and more liquefied and less and less textured with changes in its rheology. It is partly visible with a gain of approximately 7.5% of fine particles/dry mass between the first and the seventh circulation of the SC bioextrudate. Moreover, after 7 re circulations they observed twice the amount of fine particles with than without adding enzymes (Table 2c). It emphasizes the actual enzymatic activity during the extrusion process, while underlining the short re sidence time. Indeed, after a single circulation no real difference is visible with or without the addition of enzymes. This particle short ening phenomenon isn't observed when recirculating without enzymes. Samaniuk et al. (2011) observed a weight average fiber length reduc tion of 0.3 mm between 5 min and 30 min of reaction. Moreover, after 15 min of reaction the bioextrudate is described as a homogeneous paste comparable to toothpaste with a torque value close to zero. A slight increase in the sugars released is also observed after several re circulations (Table 2c). A maximum of 4% more total sugars/dry mass is already observed after the fifth recirculation of the SC, whereas a greater cellulose conversion, up to 50% more than the same unmixed enzymatic hydrolysis, is measured after 30 min in the torque rheometer. When compared with a 70 min enzymatic reaction in a batch reactor, the recirculation technique shows the same reducing sugar ratio re garding the dry mass, but almost twice the ratio of free sugars and 2% more of total sugars.

Bioextrusion of lignocellulosic materials appears to improve the biomass deconstruction and the sugar release in the few existing examples. Low sugar yields observed directly after the bioextrusion or during a comparable reaction time with the torque rheometer, confirm that these conditions are not sufficient for a good saccharification of the biomass. However differences are more visible after some time, either by prolonging the bioextrusion or incubating the bioextrudate into di luted batch conditions. It indicates that bioextrusion pretreatment in itiates a good saccharification that requires longer reaction times to be completed. Mechanisms behind these results have to be studied in more details.

3. Future prospects

3.1. Process adaptation to different biomass characteristics

Numerous factors, influencing the pretreatment, depend on the biomass characteristics before extrusion, such as moisture content, substrate composition and particles size. Influence on the bioextrusion process efficiency of some of these characteristics is not clearly defined yet. For example, Xu et al. (2015) showed that there was no significant influence of the particle size reduction in the case of rice bioextrusion, whereas for Karunanithy and Muthukumarappan (2013) it appears critical for the feeding step. However, it is clear that adaptations of the extrusion pretreatment process to different inputs should be in vestigated. Indeed, a bio refinery plant may be supplied by different farms, different biomasses, depending on the time of the year, climatic conditions, production methods. Vandenbossche et al. (2016) used six different lignocellulosic biomasses, sweet corn, sugarcane bagasse (*Saccharum officinarum*), eucalyptus (*Eucalyptus grandis*), vineyard pruning (*Vitaceae vitis*), blue agave bagasse, oil palm empty fruit bunch. Is a good example of the different process adaptations that have to be made (particle size, screw speed, restrictive screw elements length, li quid/solid ratio…).

Because of storage and manipulation limitations, research and de velopment over biomass extrusion pretreatment is usually done with dry biomass. This dry substrate is then rehydrated in order to test dif ferent moisture contents. Furthermore, very low moisture contents lead to low results and processing difficulties (Karunanithy and Muthukumarappan, 2013). Hence, in the perspective of an industrial bio refinery production, it could be more interesting to use fresh feed stocks obtained directly after harvesting than to add water during the process. This could reduce drying cost and time.

3.2. Complete substrate valorization

Biomass is a very complex substrate composed of various polymers and families of molecules that are closely linked together. The release of one component often requires the removal of others. Moreover, optimal valorization of the biomass is the key of the bio refineries economic viability. Thermomechanical pretreatment is a good tool to deconstruct matrices, disrupt cellular barriers and increase the substrate accessi bility. Whereas enzymes, in its large diversity can specifically catalyze the breakdown of large molecules, and thus help to extract and separate various components like proteins, sugars, lipids and lignins. The work of de Mesa Stonestreet et al. (2012) and Xu et al. (2015) are in this

regard very interesting. They measured the protein and other com pounds concentration while hydrolyzing starch with a bioextrusion pretreatment step. The de Mesa Stonestreet et al. (2012) bioextrusion of sago starch gave similar mechanical behaviors than those obtained by Govindasamy et al. (1997b). They also observed higher protein content and, for most of it, higher digestibility than the sole batch liquefaction. Xu et al. (2015) measured amino acids contents 1.7 1.8 times higher than with other pretreatment, while increasing the gelatinization of the starch. This increase was attributed to a better starch deconstruction that released more amino acids.

Moreover, traditional pretreatments intensive conditions, with high physical and chemical constraints, lead to higher biomass deconstruc tion but also to some vulnerable substances loss (e.g., polyphenols, flavonoids and vitamins). Xu et al. (2017, 2015) observed better phe nolic compounds and soluble solid content of other compounds re covery with bioextrusion. The hypothesis was that the mild but efficient material deconstruction allowed to liberate more secondary substances without degrading it.

3.3. Research and development to come

Comparison, between the level of information available on starch bioextrusion and on lignocellulose bioextrusion, underlines a gap in knowledge where resources should be directed. Bioextrusion of lig nocellulose has only started a few years ago, and more results and publications are needed to analyze the catalysis of complex biomasses with complex enzymatic cocktails. As shown by the numerous pub lications on starch bioextrusion, statistical analysis, such as response surface methodologies, are powerful tools to analyze and understand processes involving many parameters. These kind of analysis should be used to measure the influence of the different factors on the bioextru sion of lignocellulose. Moisture content and enzymatic loading should be considered, as it is underlined by the starch bioextrusion publica tions.

Extrusion can easily be brought to an industrial level and this is one of the main interests of this technology. Methods enabling the pro duction of continuous sugar syrups have been patented (Ogawa et al., 1994; Su et al., 2014).

Ogawa et al. (1994) proposed a method of bioextrusion in order to work with high consistency media without any subsequent concentra tion process. They claim a technic allowing the continuous production of concentrated sugar solution with major energy consumption savings. Su et al. (2014), added a concentration technique to the bioextrusion to produce different concentration of starch syrups. However, using di rectly broken rice, they indicate a production cost reduction thanks to reduced reaction time and improved yields. Second generation bior efineries could also be a place of interest to develop the bioextrusion of lignocellulosic materials. To this matter, Vilarem et al. (2014) pub lished a patent that include the bioextrusion step following a classical reactive extrusion step and preceding a longer batch fermentation. This method is designed in order to produce ethanol from the released su gars. This shows an easy and interesting transfer to the industrial scale. However, some technical issues have yet to be addressed, such as the connection in between the extrusion and the batch step.

4. Conclusion

Bioextrusion has been repeatedly proved to enhance the yield of the subsequent enzymatic batch reactions with high solid content condi tions. This is probably linked to the good mixing conditions between the substrate and the catalyst during the process. Extrusion process unique flexibility allows an easy adaptation of the process conditions, and offers the possibility to combine several pretreatments. Among all the tested factors, enzyme concentration and moisture content are the most influencing factors. The short residence time doesn't allow com plete reactions to take place but offers a good pretreatment method to initiate enzymatic reactions with limited use of solvents.

Declarations of interest

None.

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