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Impact of wet aerobic pretreatments on cellulose accessibility and bacterial communities in rape straw

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HIGHLIGHTS

- Rape straw is a potential feedstock for bioethanol and anaerobic digestion sectors.
- A three-days aerobic incubation could improve the cellulose accessibility of the studied straw.
- A biological pretreatment of straws for increasing cellulose accessibility is proposed.

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ABSTRACT

A new pretreatment method of lignocellulosic biomass was explored by using a wet aerobic process with an alkaline lignin and a mineral salt solution. This treatment significantly improved structural modification of rape straw used as substrate model in this study. Change in cellulose accessibility to cellulase of rape straw rose up to six fold within the first days of this pretreatment without generated significant modification of van Soest lignocellulose fractionation. The biological pretreatment apply to rape straw induced a high microbial activity revealed by quantitative PCR and sequencing techniques, suggesting that bacteria including *Xanthomonadales* and *Sphingobacteriales* may be involved in this lignocellulosic biomass transformation. Moreover, results of this work demonstrate that the endogenous microbial community associated with rape straw plays a key role in its alteration.

1. Introduction

Valorization of lignocellulosic wastes is a promising solution to the growing demand for renewable energy and the production of value-added sugars (Teghammar, 2013). Oilseed rape (*Brassica napus*) is widely cultivated for the extractable oil in its seed which accounts for about 18% of total dry weight of the whole plant. Rape straw is a particularly attractive agricultural residue for methane or biofuel production because of its high cellulose and hemicellulose content. Rape plant cultivation is often geographically concentrated, thereby facilitating harvest and transport of the material for processing (Fan et al., 2014). However, due to the lack of an effective application, until now, on most farms, rape straw is left in the field. With a production over 38 million tons per year in the European Union (Romero et al., 2015), this agricultural residue is thus a potentially useful lignocellulosic substrate that is readily available for the production of energy.

The biodegradation of lignocelluloses is difficult due to their compact structure and the presence of lignin, a recalcitrant heteropolymer of aromatic compounds (Crawford and Crawford, 1976). In the plant cell wall, cellulosic fibers present a crystalline structure organized in microfibrils (Cheng et al., 2011). Lignin covalently links with hemicellulose and cellulose microfibril aggregates to form macrofibrils, making the whole structure compact and inaccessible to hydrolytic enzymes (Thygesen et al., 2012). Some methods enable estimation of the cellulose accessibility of lignocellulosic substrates. Classical techniques include nitrogen adsorption, mercury porosimetry and solute exclusion (Meng and Ragauskas, 2014). The drying process involved in nitrogen adsorption and mercury porosimetry causes irreversible collapse of pores that could bias results (Meng and Ragauskas, 2014). Solute exclusion, which estimates the accessibility of cellulose by the pore characteristics, is based on the hypothesis that biomass pores are the same shape (Park et al., 2006). Structural changes in

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lignocellulosic biomass have also been studied by X-ray Diffraction (XRD), Fournier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) (Motte et al., 2015). These non-destructive techniques allow two- or three-dimensional observations of the lignocellulosic surfaces. However, measurements made with these direct techniques are mainly limited by their small field of observation that cannot provide a comprehensive view of the material. Another promising approach consists in determining cellulose accessibility to cellulase (CAC) using a recombinant protein comprising a cellulose binding module of a bacterial cellulase, and a fluorescence module (Hong et al., 2007). The advantage of this approach is that the recombinant protein is very similar in size to a bacterial cellulase, which may provide a more accurate estimation (Gao et al., 2014).

Recent research investigated a wide range of pretreatment methods aimed at increasing accessibility to cellulose in lignocellulosic substrates, either by degrading the lignin polymer or by destroying the plant cell wall ultrastructure (Mosier et al., 2005). Among them, physical pretreatments such as grinding, extrusion and steam explosion have been thoroughly studied and applied at lab-scale (Carrere et al., 2016). Basic grinding decreases the volume of feedstock and significantly increases the accessibility of the cellulose in lignocellulosic biomass (Monlau et al., 2013), but the amount of energy required for grinding large feedstocks such as crop residues is not negligible (Dumas et al., 2015). The same problem applies to steam explosion. Chemical pretreatments consist in exposing the biomass to acid, alkali, solvents or oxidants (Carrere et al., 2016). However, the use of chemicals in large scale facilities might have economic and ecological limitations. Therefore, the development of an efficient yet sustainable pretreatment method is still urgently required for the valorization of lignocellulosic biomass.

Biological pretreatments of lignocellulose consist in using enzymes and/or microorganisms. This approach is sustainable and eco-friendly. Among these processes, enzymatic pretreatments can involve application of cellulase, xylanase, pectinase as well as lignin-degrading oxidases such as laccase (Quéméneur et al., 2012). The major risk of this method is that the released sugars are rapidly consumed by endogenous microorganisms. Pretreatment goals can also be achieved by the activity of some microorganism themselves, especially fungi and bacteria, which are natural decomposers (Eastwood et al., 2011). However, the process generally lasts for at least two or three weeks. In the meantime, the introduced fungal strain often consumes lignin as well as polysaccharides, causing the considerable loss of readily accessible cellulose (Wan and Li, 2012).

The role of bacteria in lignin degradation has been recently revealed by the identification of bacterial peroxidases (Ahmad et al., 2011). Lignin-degrading bacterial strains have also been isolated from the environment (Taylor et al., 2012; Tian et al., 2016c). Compared to fungi, bacteria generally present a higher growth rate, a more active metabolism, and a higher resistance to environmental changes (Bugg et al., 2011). The highly active metabolisms of bacteria might thus open the possibility of much shorter pretreatments than with fungi. Moreover, a wide range of genetic tools is already available for bacteria, facilitating their genetic modification. The use of bacteria for the pretreatment of lignocellulosic biomass thus appears as plausible.

The objective of this study was to obtain insight into the impact of the activity of ligninolytic bacteria on the accessibility of rape straw cellulose during a short aerobic (3 to 11 day) pretreatment. To achieve this objective, high concentrations of isolated ligninolytic strains were added to rape straw. These strains were firstly enriched in a medium containing Kraft lignin. The effect of the medium only (without bacteria) was also considered as control, in order to evaluate its effect in promoting the lignin degradation activity of the endogenous population.

2. Materials and methods

2.1. Design of the leach bed reactor (LBR)

Ten double-walled LBRs were constructed in polyvinyl chloride (PVC) tubes (Fig. S1). Each reactor had an internal diameter of 15 cm, a height of 30 cm and a total capacity of 5.3 L. The reactors were divided into two parts by a poly(methyl methacrylate) (PMMA) grid: the upper part contained the solid phase substrate (rape straw) and the bottom part held the liquid phase. The temperature inside the reactor was controlled by constant water flow between the two walls (3 cm in thickness), which was maintained by a recirculating chiller (model Polystat, Thermo Fisher scientific, USA). Each LBR was equipped with an automated pumping device which enabled the recirculation of the liquid phase in the reactor at the desired rate. pH and oxidation reduction potential (ORP) were continuously monitored by probes (Consort, Belgium) fixed to the leachate recirculation lines. Liquid sampling valves were located at the bottom of each LBR to collect leachates. Each LBR was equipped with an air injection system to allow the injection of compressed air at the desired rate during the pretreatment process.

2.2. Experimental setup

The wet pretreatments of rape straw were carried out in aerobic leach bed reactors (LBRs). Pretreatments were carried out in batch triplicate at 30 °C. The process involved an aqueous solution (or suspension) that was continuously spread over the surface of the straw by a recirculation system which pumped it from the bottom to the top of the LBR. The ligninolytic strains were inoculated by adding high concentration liquid culture, while the equivalent volume of sterile solution was added to the non-inoculated controls (Table 1). In each LBR, 50 g of cut rape straw and 400 mL each of inoculated or non-inoculated solution (the liquid phase) were added. The liquid phase was continuously recirculated at a rate of ca. 30 L/h. The aerobic condition (oxidation reduction potential (ORP) > 200 mV) inside the LBR was maintained by air injection. Air was continuously injected at ca. 25 mL/min. The pretreatments lasted for 11 days. Samples of the solid and liquid phases were collected at 3, 5, 7, and 11 days. The effect of wet pretreatments was first measured by cellulose accessibility to cellulase (CAC) assay. A CAC assay was performed on solid samples on each sampling occasion, whereas van Soest fractionation of the solids, qPCR and Next Generation Sequencing for microbial community analysis (NGS) of solids and liquid were only performed on samples on the 3rd and 11th day. Statistical significance was calculated by the *t*-test.

2.3. Raw material and chemicals

Rape straw stems (*Brassica napus*) were harvested near Rennes (Nouvoitou, (48.03, −1.54), Brittany, France) and stored in a dry,

Table 1
Bacterial cultures and sterile solutions used for pretreatment of rape straw.

Name	Bacteria inoculated	Lignin	M9
Ser_lig_M9	*Serratia* sp.	+	+
Pseu_lig_M9	*Pseudomonas* sp.	+	+
Steno_lig_M9	*Stenotrophomonas* sp.	+	+
lig_M9	–	+	+
lig_NaCl	–	+	– (NaCl)
M9	–	–	+
NaCl	–	–	– (NaCl)

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