Fungal pretreatment of wheat straw for anaerobic digestion

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Abstract
Recently, there has been a considerable interest for the production of renewable energy from lignocellulosic biomass which presents several advantages. It is, however, a technological challenge because of the difficulty to hydrolyze lignin contained in this biomass. This limitation can be overcome by pretreatments. Among them, low-cost fungal pretreatments are attractive. This study focuses on the selection of a fungal strain, in order to increase methane production from wheat straw. After a screening on 176 strains, thirteen were preselected and used to pretreat straw. BMP of those straws were measured. Compared to the control straw and considering pretreated VS, up to 43% more methane were obtained. Taking into account the dry weight loss observed during the pretreatment in non-optimized conditions, it was found up to 21% more methane compared to the control straw.

Keywords
Anaerobic digestion; biogas; pretreatment; white-rot fungi; lignocellulose

INTRODUCTION

Lignocellulosic biomass (LB) is a choice substrate for energy production (Wan & Li, 2012). Anaerobic digestion of agricultural residues such as straw has a growing interest because it generates a limited competition with food crops for land use. LB is principally constituted by cellulose, hemicelluloses (carbohydrates) and lignin (polyphenols). Lignin hydrolysis is difficult and it is poorly biodegradable under anaerobic conditions. Consequently, pretreatments are necessary to facilitate the access to LB fermentable sugars (Monlau et *al.*, 2013).

Pretreatment, especially physical (grinding…) and thermo-chemical (alkali, acid…) can consume up to half of the energy used during the processing of the substrate. Thus, it is an expensive step representing a significant barrier to their full-scale implementation. Biological pretreatments (fungi, enzymes...) have significantly less drawbacks. In particular, the use of fungal pretreatments enables economical gain by low energy requirement. Fungi have environmental benefits with low inputs and outputs (inhibitors and wastes). Solid state fermentation process (SSF) which allows higher feedstock loads than liquid culture must be preferred (Tian et *al.*, 2012). An efficient fungal pretreatment must, among others, limit the hydrolysis of cellulose and hemicelluloses to avoid methane potential loss.

The substrate chosen in this study for biogas production is wheat straw since it is an experimental model because the composition of the organic matter (VS) is representative of herbaceous biomass (Vassilev et *al.*, 2012).

The objective of this study was to select the most efficient strain for the selective delignification of straw (low sugars consumption and weight losses). The study focused on basidiomycete strains, the most efficient lignocellulose degraders among fungi (Hammel, 1997; Sánchez, 2009).

A first miniaturized screening allowed comparing 176 strains for their performance in lignin, cellulose and hemicellulose degradation and sugars accessibility to enzymes (Zhou et *al.*, submitted in 2014). Thirteen strains were preselected, they belong to White-Rot Fungi that are considered as most efficient organisms for delignification (Hatakka, 1983; Müller & Trösch, 1986; Wan & Li, 2012). The best Brown-Rot Fungi was also kept (*Gloeophyllum trabeum*, strain E). This paper presents the preselected strains performances in pretreatment for anaerobic digestion of wheat straw.

MATERIAL AND METHODS

Fungal pretreatment

Table 1. Preselected strains

	Strains	Added glucose amount (*mg/g TS straw*)
E	*Gloeophyllum trabeum*	200
D	*Ganoderma adspersum*	200
B	*Trametes suaveoleus*	200
G	*Trametes cingulate*	200
F	*Polyporus brumalis*	200
H	*Phlebia sp.*	200
N	*Trametes ljubarskii*	50
M	*Trametes membranacea*	50
L	*Dichostereum effuscatum*	50
A	*Trametes pavonia*	50
J	*Leiotrametes sp.*	50
K	*Ganoderma flaviporum*	50
I	*Trametes menziesii*	50

SSF in deep well. Thirteen preselected strains (Table 1) were cultivated in three series (occurring at different times in the year). Pretreatments were carried out with three deep wells per strain (100 mg of straw/well in triplicate). Sterilized straw, ground to 4 mm was inoculated with one 5-mm diameter agar disc of 7-days-old mycelia. A nutrient solution (starter solution) was added. This addition of a carbon and nitrogen source was to ensure initial fungal growth. For the first set, 200 mg glucose/g dry straw and 18.4 mg of tartrate diammonium/g TS were added. In contrast, these quantities were divided by four for series 2 and 3, in order to reduce proportion of methane derived from the addition of nutrient solution.

The straw was then pretreated under a constant humidity during 12 days at 25°C. Measurements of biochemical methane potential (BMP) were carried out independently for each series. A control (T) was made: wheat straw treated under the same conditions but without fungal inoculation.

SSF in columns. Other samples were obtained with a greater amount of substrate in SSF glass columns. A column contained 20 g of ground wheat straw dry matter (TS), 25 mg of glucose/g TS and 2.5 mg of tartrate diammonium/g TS. Each column was inoculated with 120 mg dry ground mycelium. Columns were thermostated at 28°C. The initial water retention of the straw was 90%. Airflow saturated with moisture was set to 120 mL/min, measured by a ball flowmeter. For each strain, cultures in SSF columns were tripled and the obtained pretreated wheat straws were homogenized. A control (T_{col}) was also made: SSF culture without inoculation.

Biochemical Methane Potential (BMP) measurements

Methodology. BMP of pretreated straws were measured to obtain the maximum amount of methane from these substrates. Straws were digested anaerobically in batch anaerobic flasks at 36 °C. The volume of each flask was 600 mL, with a working volume of 400 mL. It contained the substrate (1.3 g TS/flask), an inoculum from anaerobic digester (≈ 3 g VS / L), water, macro- and microelements and a bicarbonate buffer to ensure optimum anaerobic digestion conditions (see Monlau et *al.*, 2012 for exact composition of medium). Duplicates were made for straw pretreated in deep well and triplicates for SSF columns. Flasks were mechanically agitated. Biogas production was followed by pressure measurements until the end of production (plateau phase). Biogas composition was obtained at each pressure reading with a micro-gas chromatograph: µGC Varian lGC-CP4900 (see Motte et *al.*, 2014 for material details). Volumes values are expressed in temperature and pressure standard conditions (NmL).

Normalization of BMP results. Because of the two different concentrations of nutrient solution and the three series of BMP measurements, results were normalized to better compare all samples. A correction of the treated straw BMP (BMP_X) was performed to erase the effect of the nutrient solution. This correction is relevant since the starter solution was transformed into fungi (high BMP) or remained on straw. In any case, it constitutes easily degradable biomass. In addition, the gap between the control straw BMP (BMP_T) and the BMP of control straw with starter corresponded to the theoretical amount of methane expected for the starter (data not shown). BMP were then normalized by the BMP of control straw to calculate the improvement ratio 'Improvement 1': Improvement 1 = (BMP_X - starter)/BMP_T; with BMP_X: BMP of pretreated straw with strain X; starter: theoretical amount of methane produced from the nutrient solution; BMP_T: BMP of the control straw; BMP_X, starter and BMP_T are expressed in NmL/g of pretreated VS.

During pretreatment there were weigh losses (10 to 30% TS depending on the strain and culture conditions). They can reduce the effectiveness of the pretreatment, especially if losses concern fermentable sugars. In order to consider these losses, BMP were expressed in NmL/g of initial TS (before pretreatment) and the improvement ratio ‘Improvement 2’ was calculated: Improvement 2 = (BMP_X - starter)/BMP_T; with BMP_X, starter and BMP_T expressed in NmL/g initial TS.

RESULTS AND DISCUSSION

Samples pretreated with SSF in deep well

Figure 1 shows the improvement of methane potential relatively to the control straw and expressed per gram of volatile matter of pretreated straws. Some pretreated straws have a ratio ‘Improvement 1’ lower than one. Therefore, the concerned strains did not improve the BMP comparing to the control straw in these culture conditions. Consumption of cellulose and hemicellulose during the pretreatment may have been too high, this hypothesis must be checked. These strains do not seem interesting for pretreatment for anaerobic digestion. Interesting pretreated straws have the highest value of the standard deviation greater than one (see black box in Figure 1). It can be observed an improvement up to 40%. If weigh losses are taken into account thanks to the ratio ‘Improvement 2’ (Figure 2), only four strains are efficient (strains A, J, K and I); up to 20% improvement was found after pretreatment.

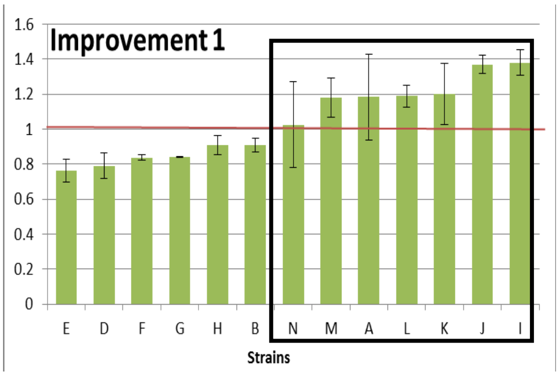

Figure 1. Ratio Improvement 1, BMP improvement reported to **pretreated organic matter** in deep well with several strains

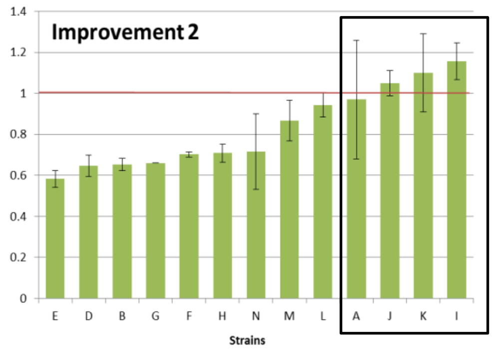

Figure 2. ratio Improvement 2, BMP improvement reported to **initial dry matter** in deep well with several strains

Strains judged as non-performing with the criterion ‘Improvement 1’ all belong to the first series (strains B to H). They had received more starter (mainly glucose) than others. According Reid and Deschamps (1991), glucose as co-substrate with synthetic lignin can limit the delignification. The greater amount of glucose received for series 1 might have limited the performance for concerned strains. Further experiments will be carried out to confirm or refute the effect of glucose on the delignification in our culture conditions. Caution is thus required to interpret results for series 1 in deep well (strains B to H).

Samples pretreated in SSF columns

To complete first analyzes, other SSF cultures were carried out at a larger scale and their BMP were measured (Figures 3 and 4). All samples were obtained with the same small quantity of starter solution.With these conditions, some strains of series 1 seem interesting like strains G and F, which seems to confirm the assumption of the negative impact of high starter concentration. BMP expressed per g of pretreated VS (Figure 3) show that pretreatment improved digestibility. For all strains, pretreated straws have a higher BMP than control straw (T_{col}). In the best case, up to 43% more methane was obtained. Weigh losses are taken into account thanks to the BMP expressed per gram of initial TS (Figure 2). This global efficiency (pretreatment and anaerobic digestion) can reach 21% more methane than control straw.

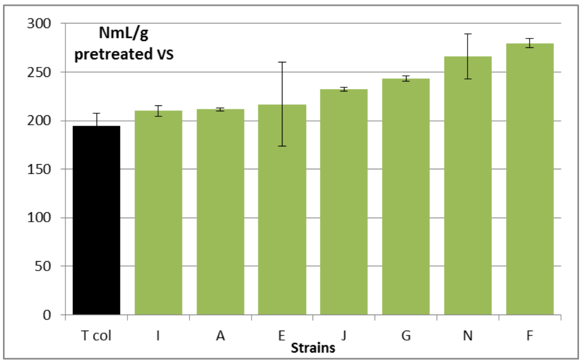

Figure 3. BMP of wheat straw pretreated in columns (NmL/g pretreated VS)

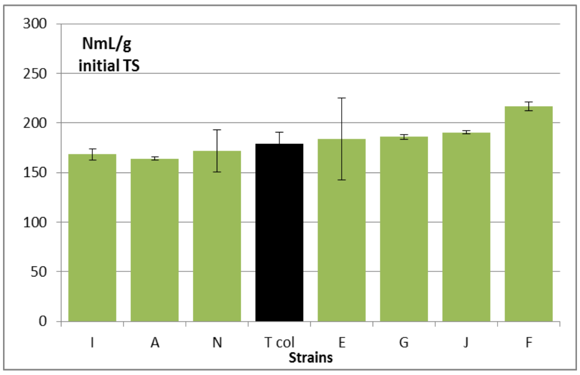

Figure 4. BMP of wheat straw pretreated in columns (NmL/g initial TS)

CONCLUSION

Following these results, *Polyporus brumalis* BRFM 985 (strain F) was selected and a French patent has been registered (Gimbert et *al.*, 2014). This strain allows a 20% increase of methane production from straw after taking into account weigh losses. An improvement of the biogas production rate would also be relevant for anaerobic digestion plants.

Further work will focus on the optimization of culture parameters (temperature, duration; moisture content, supplementation with metals) regarding the BMP of pretreated straws. Some pretreatment trials with this strain are also done in pilot reactors (400 g DM). Dry anaerobic digestion will be realized in reactors (6L) to assess the impact of pretreatment on digestion rate and methane yield in conditions closer to full-scale biogas plant.

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