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COMBINATION OF WHITE ROT FUNGI PRETREATMENT AND BATCH SOLID-STATE ANAEROBIC DIGESTION OF WHEAT STRAW

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Abstract

Impact of *Polyporus brumalis* BRFM 985 pretreatment in non-sterile pilot-reactor on the batch solid-state anaerobic digestion of wheat straw was studied. Pretreatment was carried out on 400 g_{TS} of 1-5 cm autoclaved straw inoculated with 100 g TS of colonized miscanthus pellets. Mesophilic anaerobic digestion was carried out in four 6-L bioreactors. One digester contained only inoculum (digested cattle manure) to estimate endogenous methane production which was subtracted to other results. Other ones contained 111 g_{TS} inoculum and 151.2 g_{TS} wheat straw and 37.8 g_{TS} miscanthus (Control 1); 151.2 g_{TS} wheat straw and 37.8 g_{TS} fungal colonized miscanthus (Control 2) or 189 g_{TS} pretreated wheat straw (Pretreated).

Total amount of liquid in each reactor containing a substrate was 2.3 L, composed of tap water buffered with 1.3 g/L $NaHCO_3$ and 1.2 L liquid digestate.

Methane productions from controls 1 and 2 were the same at low digestion times. Higher production from Control 1 (up to 6-7%) at high digestion times can be linked to the loss of miscanthus organic matter during pretreatment. In contrast, the methane production increased by 10-20% after straw treatment, showing an improved biodegradability of pretreated straw. However if mass losses occurring during fungal pretreatment are taken into account by referring methane yield to initial TS, pretreatment leads to a methane production decrease (-7% after 60 days and -9% after 127 days), except at short digestion time where a slightly higher amount of methane (+6% after 15 days) was recovered. In conclusion, fungal pretreatments appeared interesting to increase anaerobic digestion rate and to ease batch SSAD start-up phase but must be optimized to maximise biogas production.

1- INTRODUCTION

Farm anaerobic digestion sector is facing a fast development; with as an example 1000 farm biogas plants expected in France in 2020 whereas 185 were in operation at the end of 2014. This fast development requires the use of various feedstocks. Lignocellulosic residues such as wheat straw are available at the farm-scale and can be stored all over the year. Due to their high solid contents and their ability to float above aqueous media, they are more suited to Solid-State Anaerobic Digestion (SSAD) processes than to liquid processes [1]. SSAD processes present several advantages such as the reduction of digester volume for a given amount of feedstock and lower energy requirement to heat the digester [2]. In addition batch processes allow simpler and cheaper reactors which are more adapted to farm applications [3]. In addition, discontinuous leachate recycling improves the substrate degradation in batch SSAD reactors [4,5].

However, the structure of lignocellulosic residues and the presence of lignin limit the bioconversion of cellulose and hemicelluloses into biogas. Several kinds of pretreatments have been studied to increase the accessibility of holocelluloses for anaerobic digestion microorganism consortia [6-8]. These pretreatments are classified into mechanical, thermal, thermo-chemical and biological. The first objective of these pretreatments is to improve biogas from lignocellulosic residues is lignin degradation or break-down of lignin-polysaccharides links [9]. The main processes known for their delignification efficiency are thermal (steam explosion), thermo-chemical (mainly alkali) and biological pretreatments.

If steam explosion is applied at large full scale plants treating sewage sludge and, in a lower extent, municipal solid wastes, its implementation at smaller farm scale plants seems very difficult. Alkali pretreatments have shown good performance to increase biogas yield from various lignocellulosic feedstocks but the presence of salts (in particular sodium cation) in digestates will be detrimental for their valorization by land application which is the classical use of digestate at farm scale and represents a key environmental advantage of biogas processes. The most studied biological pretreatments of lignocellulosic biomass are enzymatic hydrolysis and fungal pretreatments. They present the advantage of low energy requirement and no chemical inputs. In the case of enzymatic hydrolysis, most of the commercial enzyme cocktails are produced by fungi, in majority by ascomycetes *Trichoderma* and *Aspergillus* genus. These cocktails are mainly composed of cellulases and xylanases. Their use requires a prior step to delignify the biomass and to make the holocelluloses accessible to enzymes. In comparison to fungal pretreatments, enzymatic pretreatments are in general, more complex and expensive as they require steps for enzyme production and extraction [10]. Indeed additional benefits of fungal pretreatment lie in their direct culture on the substrate to pretreat in solid-state fermentation processes which require low amounts of water and are consistant with further solid-state anaerobic digestion systems. Long reaction times of several weeks are often highlighted as drawback of fungal pretreatment but in the case of lignocellulosic residues this pretreatment can be carryed within the storage period which is of several months.

Among fungi, white-rot fungi (WRF) are the most efficient organisms for delignification [10]. They have a unique enzymatic system giving them the capacity to attack phenolic structures and to degrade lignin to CO_2. However, their efficiency is strain, substrate and culture conditions dependent [11]. In an earlier study carried out in small columns (20 g wheat straw) with twelve strains in sterile conditions, *Polyporus brumalis* BRFM 985 was shown very efficient since up to 43% more methane per gram of pretreated volatile solids were obtained

in BMP tests conditions. Taking into account the dry weight loss observed during the pretreatment, it was found up to 21% more methane per gram of initial total solids [11].

The objective of the present study was to move one step forward in process scaling up and to carry out fungal pretreatment in pilot-scale (400 g wheat straw, under non-sterile but clean conditions) and to assess anaerobic digestion performance in batch solid state anaerobic digester with leachate recirculation.

2- MATERIALS AND METHODS

2.1 Wheat straw pretreatment

Winter wheat straw (*Triticum aestivum*) was obtained from Vivescia (Reims, France). Wheat straw was inoculated with *Polyporus brumalis* BRFM 985 colonized miscanthus pellets. 50 g autoclaved miscanthus pellets were inoculated sterilely with 50 mL of mycelial solution. After a 24 h-incubation at 30°C in a Roux flask, 25 mL sterile mQ water were added. Then, incubation lasted 6 to 9 days more with a daily manual agitation.

The mycelial solution corresponded to a part of the ground mycelium (Ultra-Turrax T25 Blender, 9500 rpm, 1 min in 25 mL sterile mQ water) of a seven-days old sterile liquid culture (20 g/L Malt broth extract at 30°C with passive air exchange) that was inoculated with seven 5-mm diameter agar discs of ten-days old mycelia. 10 mL of the ground mycelium were added to 40 mL sterile mQ to constitute the mycelial solution.

200 g of sterile straw were inoculated sterilely with one flasks of colonized miscanthus. Per 100 g straw, 113 mL of mQ water were added before autoclaving and 255 mL after, so as 5 mL of metal solution. Metal solution (filtered at 0.2 µm) was composed of CuSO4 and FeSO4 at 18 Mm.

400 g of this straw was incubated in an aerobic (0.4-0.7 Lair/min) pilot reactor of 40 L. The reactor was not sterile even if cleaned 20 min at 120°C and the filling was also realized in clean conditions. Incubation lasted 13 days with constant moisture content (≈90%). Straw was distributed on two trays, one with raw straw (≈10 cm) and the other with scissors cut straw (1-5 cm). After the pretreatment, samples were freeze-dried. Pretreated straw used for anaerobic digestion was 1 to 5 centimeters long.

2.2 Biomethane potential (BMP)

Methane production was assessed in batch biochemical methane potential (BMP) tests in mesophilic conditions (37°C). Each sample was mixed in a 500 mL plasma bottle with the anaerobic sludge, oligoelements and phosphate buffer solutions. The inoculum used for BMP tests was a granular sludge from a mesophilic anaerobic digester treating sugar effluent. The S/X ratio of samples was equal to 1 gVS substrate/gVS inoculum. Biogas volume was measured by pressure measurement and its composition was measured by gas

chromatography [11]. A control without substrate was carried out to measure endogenous methane production. The latter was subtracted from the methane production of straw samples. Each experiment was triplicated.

2.3 Solid state anaerobic digestion

Experiments were performed in glass reactors at 37°C with a working volume of approximately 3.5 L. The mix inoculum/substrate was disposed on a grid to allow the recovery of leachate in the 1.5 L- leachate tank. This tank was connected to liquid pumps that recycled the whole leachate volume every 2 hours above the inoculum/substrate mix. The head-space (≈1L) was connected to mass flowmeter (MilliGas counter-1V3.0 PMMA, Ritter Inc., Germany) to ensure biogas volume measurements. Those data were registered automatically on a computer. Biogas composition was measured by gas chromatography [11].

Inoculum was starved digestate and leachate issued from cattle manure and wheat straw. The endogenous production from anaerobic inoculum was estimated with a reactor containing only inoculum whose amount was chosen to have similar TS of 18 % in the solid bed of all reactors. Taking account the leachate amount, overall TS was 9.4 %. A reactor containing pretreated wheat straw as substrate was prepared (Pretreated). Two reactors were used as control. The first one contained miscanthus pellets and wheat straw (Control 1) to estimate the role of pretreatment process in Pretreated. The other contained colonized miscanthus pellets (corresponding to the inoculum for straw pretreatment) and wheat straw (Control 2) to distinguish the part of aerobic inoculum compared to Control 1 and the methane production only due to wheat straw modification by comparison with Pretreated. All substrates were freeze-dried and because wheat straw and miscanthus pellets were autoclaved before fungal pretreatment, non-inoculated substrates were also autoclaved before before freeze drying. Substrate/inoculum ratio was 3 based on VS basis [12].

Miscanthus proportion compared to wheat straw in reactors was representative of the one used during pretreatment. Wheat straw amount in Control 1 was chosen to obtain the same total substrate VS than in Pretreated. This amount was also used in Control 2 but total substrate VS was a bit lower in this reactor (due to different VS amounts between colonized and uncolonized miscanthus). The 3 reactors contained 300 ± 5 g_{TS} including 111 g_{TS} (54 g_{VS}) inoculum and 189 ± 5 g_{TS} of substrate, corresponding to 178 g_{VS} for pretreatred straw, 141g_{VS} of straw and 36.7 g_{VS} of miscanthus for Control 1 and 141 g_{VS} of straw and 29.7 g_{VS} of colonized miscanthus for Control 2. Liquid inoculum (1200 mL) and tap water (1160 mL) were buffered with 1.3 g/L $NaHCO_3$ and added in each reactor. Considering digestate humidity, total volume of liquid in reactors was 2877 mL.

2.4 Analytical methods

Total Solids (TS), Volatile Solids (VS), were determined according to the APHA standard method [13]. Total nitrogen (TKN) and ammonium nitrogen ($N\text{-}NH_4$) were analysed by the

Kjeldhal method (Buchi autoKjeldhal unit K370) according to standard methods [13]. Total organic carbon (TOC) was analysed with a Carbon TOC-V module (Shimadzu). Volatile fatty acid (VFA) concentration was determined by gas chromatography.

3- RESULTS AND DISCUSSION

3.1 Biomethane potential (BMP) of substrates

Biomethane potentials of substrates used in further SSAD reactors are shown in table 1. Results show that fungi growth led to a slight decrease of BMP of both miscanthus pellets and wheat straw. This unexpected result might be explained by the consumption of carbohydrates during fungal pretreatment. Whereas the main objective of fungal pretreatment of lignocellulosic biomass is lignin degradation, some hemicelluloses and cellulose losses also occur and fungal pretreatment efficiency is linked to the capacity of fungi to selectively degrade lignin. Thus in the present study carbohydrates degradation was the major part of volatile solids degradation for both miscanthus pellets and wheat straw. This is not surprising in the case of miscanthus pellets because fungal growth was carried out to obtain a large amount of fungal biomass. However, the present results on pretreated wheat straw disagree with our earlier study where *Polyporus brumalis* BRFM 985 pretreatment led to 43% more methane per gram of pretreated VS [11]. This pretreatment was carried out in sterile conditions in small columns (20 g wheat straw) and for 21 days. In consequences, the pilot plant conditions for fungal pretreatments were less favorable for further anaerobic digestion, in particular non-sterile conditions and too short pretreatment duration might have had a negative impact. Nevertheless, it will be interesting to study the kinetic of pretreated organic matter in solid state anaerobic digesters and biogas production reported to initial amount of straw (taking into account mass losses during fungal pretreatment) which are presented in the next sections.

	BMP (NmL/gVS)
Miscanthus pellets	197 ± 4
Colonized miscanthus pellets	185 ± 3
Wheat straw	247 ± 8
Fungal pretreated wheat straw	222 ± 8

Table 1: Methane potential of substrates used in SSAD

3.2 Biodegradation of substrates in solid state anaerobic digestion

Solid state anaerobic digestion experiments were carried out for 127 days. Methane production reported to the amount of pretreated volatile solids at different digestion time is shown in Table 2. As expected from BMP measurements, final methane yield of Control 2 with colonized miscanthus pellets was lower than the one of Control 1 with raw miscanthus pellets. However, final methane yield of pretreated wheat straw was very slightly higher than

the both controls ; but the most interesting point is the improvement of anaerobic degradation rate. Indeed, after 15 days of anaerobic digestion 50% of expected methane was produced from fungal pretreated straw against 37-38% for controls. If we consider 60 days anaerobic digestion, which is the classical duration for full scale processes, 90% of expected methane production were obtained for pretreated straw against 77% for controls. In addition, after 60 days, methane yield of pretreated straw was 10% higher than for both controls.

	Control 1		Control 2		Pretreated	
Day	**Metane yield** (NmL/gVS_{pret})	**% of BMP value**	**Metane yield** (NmL/gVS_{pret})	**% of BMP value**	**Metane yield** (NmL/gVS_{pret})	**% of BMP value**
15	85	36	88	37	110	50
30	146	62	146	62	161	73
60	182	77	181	77	204	92
75	196	83	196	83	222	99
127	230	97	215	91	254	114

Table 2 Methane yields in batch reactors reported to pretreated VS after different digestion times

3.3 Methane production reported to initial straw amount

During pilot-scale fungal pretreatment, 19 % mass losses occurred in the mixture of wheat straw and miscanthus pellets. Pretreatment efficiency should thus be evaluated by expressing methane yield per amount of initial substrates. Results are shown in Table 3. Except for the 15 first days of anaerobic digestion where 6 % methane production increase was observed, fungal pretreatment resulted in lower methane production than the controls with a loss of 7% at days 13 and 60 and a loss of 9% at 127 days. These results highlight the necessity to optimize fungal pretreatment conditions, in particular pretreatment time in order to limit mass losses and to degrade lignin rather than hemicelluloses and cellulose. Indeed during pretreatment with *Polyporus brumalis* BRFM 985 in lab-scale sterile conditions for 21 days, 13.7% mass losses and 21 % increase of BMP reported to initial wheat straw amount were observed [11].

Day	**Control 1** (NmL/gTS_{init})	**Control 2** (NmL/gTS_{init})	**Pretreated** (NmL/gTS_{init})
15	83	83	88
30	137	137	127
60	173	171	161
127	219	203	200

Table 3 Methane yields in batch reactors reported to initial TS after different digestion times

3.4 Possible interest of fungal pretreatment at full-scale biogas plant

If we consider a given amount of initial wheat straw, e.g. 6 tons TS (5.68 tons VS) per year, with 19% mass losses during pretreatment, pretreated straw amount will be 4.86 tons TS and 4.6 tons VS per year. Considering the same substrate/inoculum ratio (S/X=3) as in this study, 6.1 and 4.8 batch trials per year (corresponding of digestion times of 60 and 75 days) will be carried out for wheat straw and fungal pretreated straw, respectively. In these conditions, expected methane yields are 196 and 222 Nm^3/tVS (Table 2), which corresponds to 1028 and 1021 Nm^3 annual production for wheat straw and pretreated wheat straw, respectively. Fungal pretreatment of wheat straw carried out in this study conditions is definitively not interesting for methane production from a limited amount of straw.

On the other hand, if we consider a given batch digester operating with 60 days digestion time and S/X=3 and fed with 5.68 ton VS per year, wheat straw amount being non-limited, annual methane production would be 1028 and 1159 Nm^3 annual methane production for wheat straw and pretreated wheat straw, respectively. In this case, fungal pretreatment may lead to 13% increase in biogas production.

It is clear that these results show that non-optimized fungal pretreatment of wheat straw has no or little interest considering methane production after long digestion time but the main positive result of this experiment is the enhancement of digestion rate. This point is of high interest in batch processes since low methane content in biogas during the start-up phase may hamper biogas valorization in CHP units or biogas upgrading systems.

In addition, start-up may be a critical step in batch anaerobic digestion with the risk of volatile fatty acids (VFA) accumulation and pH drop, which may lead to anaerobic digestion process failure. In our experiment, VFA maximum concentration in leachates were 5.9; 7.8 and 4.5 g/L corresponding to minimum pH values of 6.2; 5.6 and 6.05 for Control 1, Control 2 and pretreated straw, respectively. None of the reactors underwent any failure but fungal pretreatment led to the lowest accumulation of VFA and it can then be concluded that the pretreatment allowed an easier start-up phase. Indeed, accumulation of VFA during start-up phase of batch anaerobic digestion generally originates from easily accessible organic matter. This organic matter was certainly consumed during the pretreatment step.

	Control 1	**Control 2**	**Pretreated**
NH_4^+ in leachate (mg/L)	5.52	9.20	18.26
TKN in solid digestate (%TS)	2.40	2.44	2.36
TC in solid digestate (%TS)	44.93	44.88	42.97

Table 4: Ammonium concentration in leachate and carbon and nitrogen content of solid digestates at the end of experiment.

Finally, nitrogen and total carbon analysis of the three digestates were carried out at the end of experiment (Table 4). The most significant difference lies in ammonium concentration in

the leachate with the highest value in pretreated wheat straw leachate which may be due to fungi proteins degradation. This assumption seems to be confirmed by the higher ammonium concentration in Control 2 (containing colonized miscanthus pellet) than in Control 1 (containing uncolonized miscanthus). Total carbon of pretreated straw digestate was slightly lower than the controls, indicated slightly more stabilized organic matter.

4- CONCLUSIONS

Although carried out in suboptimal conditions, fungal pretreatment with *Polyporus brumalis* BRFM 985 led to the increase of methane production reported to pretreated VS of wheat straw in SSAD when short digestion time are considered (lower than 60 days). However , methane production reported to initial amount of wheat straw was lower after pretreatment, due to mass losses during fungal pretreatment. Nevertheless the most interesting results of *Polyporus brumalis* BRFM 985 pretreatment are the enhancement of the digestion rate combined with and easier start-up phase with less volatile fatty acids accumulation.

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