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White-Rot Fungi pretreatment of lignocellulosic biomass for anaerobic digestion: Impact of glucose supplementation

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ABSTRACT

Anaerobic digestion of lignocellulosic biomass is one of the most efficient ways to produce renewable energy. However, lignin contained in this biomass is difficult to hydrolyse. Pretreatment can help to overcome this limitation. Among them, low-cost fungal pretreatments seem noteworthy. Although widely used in pretreatment for bioethanol production, rot fungi have rarely been applied for improving biogas production during anaerobic digestion of lignocellulosic biomass. The present study investigates the possibility to increase methane production from wheat straw pretreated with several fungal strains. After screening sixty-three strains, twelve preselected strains were used to pretreat straw. Compared to the control straw, up to 43% more methane per gram of pretreated volatile solids were obtained with *Polyporus brumalis* BRFM 985 strain. Taking into account the dry weight loss measured during pretreatment in non-optimized conditions, up to 21% more methane per gram of initial total solids was observed. Glucose addition during the pretreatment also proved to limit delignification and thus methane production from the substrate.

1. Introduction

Renewable energy has become essential to face depletion of fossil resources and current environmental challenges such as global warming, land use, etc. Biogas production by anaerobic digestion presents several advantages. Among them, the final solid residue can be valorized as fertilizer, while produced methane offers a number of applications (heat, electricity, biofuel). According to Börjesson & Mattiasson [1], anaerobic digestion of lignocellulosic residues appears to be the most environmentally friendly way to produce biofuels.

Lignocellulosic biomass is renewable, widely available and rich in complex carbohydrates (55–75% in total solids (TS)); it is thus a choice feedstock for energy production [2]. Substrates used for anaerobic digestion should not enter into competition with food or feed crops for the exploitation of limited agricultural land resources; this is why agricultural residues such as straw are receiving growing interest. Cellulose, hemicelluloses (carbohydrates) and lignin (polyphenols) are the main compounds of lignocellulosic biomass. In anaerobic conditions, lignin is poorly biodegradable because its hydrolysis is difficult. Furthermore, carbohydrates are hardly accessible for anaerobic digestion owing to interactions and linkages with lignin. Chandler and Jewell [3] showed that one percent lignin decreased organic matter digestion by about 3%. Consequently, pretreatments have become essential for fermentable sugars from lignocellulosic biomass [4] to be more easily recovered.

Most studied pretreatments, including physical (grinding, etc.) and thermo-chemical (alkali, acid, etc.) processes are expensive and can require high amounts of energy [5,6]. Biological pretreatments (fungi, enzymes, etc.), which are considered as more environmental friendly, seem more promising. In comparison to enzymatic pretreatments using either ligninolytic enzymes [7] or cellulases, xylanase and pectinases [8], fungal pretreatments imply more simple processes because fungi can grow directly on the substrate that is to be pretreated. They therefore do not require any extraction step for enzyme recovery. Fungi have environmental benefits with low inputs (energy, chemicals) and outputs (inhibitors and wastes). However, their main drawback is the duration of the pretreatment period (usually several weeks) that should allow for a sufficient fungal population to be established before the substrate can be sig-

Abbreviations: BMP, Biochemical Methane Potential; BRF, Brown-Rot Fungi; BRFM, Banque de Ressources Fongiques de Marseille, Bank of Fungal Ressources of Marseille; HCA, Hierarchical Cluster Analysis; HPLC, high performance liquid chromatography; RI, refractive index; SSF, solid-state fermentation; TS, total solids; VS, volatile solids; WRF, White-Rot Fungi.

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nificantly altered. Fungal pretreatment during part of the storage period can circumvent this disadvantage [9].

Basidiomycete strains are the most efficient lignocellulose degraders among fungi [10,11]. In this phylum, Brown- and White-Rot Fungi (WRF) are able to attack lignin. While Brown-Rot Fungi (BRF) attack cellulose and hemicelluloses with only small lignin modifications [11], WRF can lead to important losses in lignin. Thus, WRF have been identified as the best delignifying organisms. Their ligninolytic enzymatic system is unique and based on oxidations [2,12,13]. Since they also have a hydrolytic system acting on cellulose and hemicelluloses, a selective delignification must occur [2]. If not, methane production would decrease because the carbohydrates consumed during fungal pretreatment would not be available anymore for conversion into biogas by anaerobic digestion.

It is a well-known fact that WRF can improve enzymatic hydrolysis and its subsequent sugar yield. For this reason, they have been studied with the purpose of pretreating substrates, principally for bioethanol production [2,14], although more rarely for anaerobic digestion [15]. WRF can also be used for their role in detoxification: indeed, the methane production of the substrate is higher owing to lower inhibitor concentrations. For example, before the use of anaerobic digestion, Fountoulakis et al. [16] used WRF to reduce toxic phenolic compounds in olive mill wastewater and Srilatha et al. [17] for limonene reduction in orange processing wastes. Their delignifying action can also lead to better methane production, especially on woody biomass. Amirta et al. [18] observed a 6-fold increase in CH_4 for fungal-pretreated Japanese cedar wood, compared to untreated wood (almost no CH_4). To our knowledge, this is the best improvement obtained with WRF pretreatment for anaerobic digestion.

Solid State Fermentation process (SSF) has been considered preferable to submerged fermentation for pretreatment. It allows for higher feedstock loads, favors the attachment of fungal enzymes to the substrate, as well as oxygen diffusion. Costs are lower than with liquid culture because SSF requires less aeration, heating, mixing and water [19]. The efficiency of fungal pretreatments is not only strain- and substrate- dependent: culture conditions can also have a strong influence [2].

The substrate chosen for biogas production in this study was wheat straw since it is: (i) an experimental model because the composition of the organic matter (VS) is representative of herbaceous biomasses [20]; (ii) a major source of crop residue in Europe and (iii) a biomass that can overcome the problem of feedstock seasonality in biogas plants.

An initial screening of 63 basidiomycete strains was carried out on wheat straw and miscanthus in an earlier study [21]. Strains were grown in presence of a starter solution composed mainly of glucose (200 mg/g TS straw) with a small amount of nitrogen (18.4 mg/g TS straw). Glucose and reducing sugars released after 72 h enzymatic hydrolysis were then measured for pretreated straws. Hierarchical Cluster Analysis (HCA) was applied to these data, resulting in a distribution throughout three groups of strains: "efficient", "no effect" and "negative-effect" strains. In the best case, the sugar yield was 62% higher than for the untreated control [21].

Twelve strains were selected for further studies: eleven White-Rot Fungi (WRF) belonging to the "efficient" group and a Brown-Rot Fungi, *Gloeophyllum trabeum* BRFM 236 (Banque de Ressources Fongiques de Marseille). The latter *G. trabeum* BRFM 236 was assigned to the "no effect group" after screening on wheat straw and miscanthus, however it proved to be the most efficient strain for releasing reducing sugars after hydrolysis when cultivated on wheat straw (data not shown).

During the next step, presented in this paper, the methane potential of wheat straw pretreated with preselected strains in different culture conditions was investigated. In particular, the impact of a starter solution during wheat straw pretreatment was examined.

Table 1
Composition of macro and micro-element solutions used for BMP-tests.

		Concentration ($g\,L^{-1}$)
Macro-element solution	NH_4Cl	26.6
	KH_2PO_4	10
	$MgCl_2, 6H_2O$	6
	$CaCl_2, 2H_2O$	3
Micro-element solution	$FeCl_2, 4H_2O$	2
	$CoCl_2, 6H_2O$	0.5
	$MnCl_2, 4H_2O$	0.1
	$NiCl_2, 6H_2O$	0.1
	$ZnCl_2$	0.05
	H_3BO_3	0.05
	Na_2SeO_3	0.05
	$CuCl_2, 2H_2O$	0.04
	$Na_2MoO_4, 2H_2O$	0.01

2. Material and methods

2.1. Fungal pretreatment

2.1.1. Fungal strains and substrate

As previously described by Zhou et al. [22], the basidiomycete strains used in this study originated from the "Centre International de Ressources Microbiennes" dedicated to filamentous fungi of biotechnological interest (CIRM-CF) and were maintained at the French National Institute of Agricultural Research (INRA; Marseille, France). Winter wheat straw (Triticum aestivum, Haussmann) was obtained from Vivescia (Reims, France) and harvested in the North of France.

2.1.2. SSF in 24-well plates

Twelve preselected strains (Table 3) were cultivated in three series, at different times of the year. Pretreatments were carried out with three 24-well plates (Whatman) per strain (100 mg of straw/well). Zhou et al. [21] precisely described all cultivation steps. In brief, sterilized straw, ground to 4 mm was washed and a starter solution was added. This addition of a carbon and nitrogen source was to ensure initial fungal growth. The first series comprised 200 mg glucose/g dry straw to which 18.4 mg of diammonium tartrate/g TS were added. In contrast, these quantities were divided by four in series 2 and 3, notably to reduce the proportion of methane originating from the addition of the starter solution.

Inoculation was performed with one agar disc of 7-day-old mycelia (5-mm diameter). The straw was then pretreated in water-saturated air for 12 days at 25 °C with passive air exchange. A control (T) consisted in wheat straw treated under the same conditions excepting for the fungal inoculation and addition of starter solution. Another control with a starter solution was also made (T starter). Samples were freeze-dried.

Measurements of Biochemical Methane Potential (BMP) were carried out independently for each series.

2.1.3. SSF in columns

In SSF glass columns, other pretreated samples were obtained with a greater amount of substrate with seven out of twelve fungi tested in deep well plates. These seven strains were the best in the "efficient" group (most different to the control) after a clustering on both miscanthus and wheat straw [21]. A Brown-Rot Fungi (*G. trabeum* BRFM 236) was also observed by way of comparison and because of a possible efficiency as already exposed. Similarly to the method previously described by Zhou et al. [22], each column contained 20 g total solids (TS) of autoclaved ground wheat

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