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Pyrolysis-GC–MS to assess the fungal pretreatment efficiency for wheat straw anaerobic digestion

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ABSTRACT

Cost-effective and environment-friendly pretreatments, such as fungal pretreatments, are required for anaerobic digestion of lignocellulosic biomass particularly because the amount of methane produced is often limited by the lignin content. Anaerobic digestibility is estimated using a Biochemical Methane Potential (BMP) test, which lasts several weeks. Since the Py-GC–MS technique is considered to be a rapid method for obtaining information on various organic components, its suitability for the study of fungal pretreatment efficiency in anaerobic digestion was investigated here, to our knowledge for the first time. In this goal, mycelium of the white-rot fungi *Polyporus brumalis* BRFM 985, untreated wheat straw and straw pretreated with different fungal strains and under different conditions were analysed with Py-GC–MS. In the mycelium pyrolysate, diverse compounds, often considered as unspecific, probably derive from proteins (toluene, etc.). A strong presence of tyrosine and phenylalanine among the fungus amino acids was also suspected.

As for pretreated straw samples, a correlation was observed between the amount of fungal biomass determined by qPCR (used as a reference method) and the sum of relative areas of toluene, styrene and ethylbenzene in the pyrograms, showing that it is feasible to estimate the fungal biomass amount on pretreated straws using Py-GC–MS.

In addition, the H/L-Py (Holocelluloses/Lignin) ratio, determined by dividing the sums of areas of pyrolysis compounds that have a polysaccharide (PS) and lignin (LIG) origin, was correlated to the BMP values of pretreated straws, thus showing that the pretreatment efficiency can be rapidly estimated with Py-GC–MS in the tested conditions.

1. Introduction

To face current challenges such as global warming, fossil fuel depletion and an increasing world population, second generation biorefineries are being developed. They are based on non-edible lignocellulosic substrates [1] that notably allow a greater net generation of energy per area of land [2]. Crop residues, in particular, limit the competition for land use with food. Among them, wheat straw is particularly available worldwide and can be considered as an experimental model [3].

However, the presence of lignin in such residues limits the yield of renewable fuels such as bioethanol or biogas [4,5]. A pretreatment step is required with lignin hydrolysis as main goal [5,6]. This step must also preserve the amount of carbohydrates since they are the substrate in the conversion to renewable fuels. Diverse non-biological pretreatments are effective [7] but costly (e.g. high energy consumption...) and can generate large amounts of waste (chemicals...). Biological pretreatment with lignin-degrading microorganisms have therefore become the object of renewed interest as they do not present these above-mentioned disadvantages. White-rot Fungi (WRF), wood degrading

Abbreviations: 2w, two weeks (pretreatment duration); ANOVA, analysis of variance; BMP, biochemical methane potential; BRF, brown-rot fungi; BRFM, Banque de Ressources Fongiques de Marseille Bank of Fungal Ressources; Ev., electron volt; GC, gas chromatography; H/L, holocelluloses/lignin; HSD, honest significant difference; NBO, nitrobenzene oxidation; LIG, lignin; MS, mass spectrometry; N. A., not available; PCA, principal component analysis; PS, polysaccharides; Py, pyrolysis; Py-GC–MS, pyrolysis-gas chromatography–mass spectrometry; RT, retention time; S. D., standard-deviation; SSF, solid-state fermentation; TS, total solids; UWS, untreated wheat straw; VS, volatile solids; WRF, white-rot fungi.

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fungi, are considered to be the most efficient organisms for delignification [5,8].

To ensure their growth on lignocellulosic substrates, WRF consume carbohydrates from holocelluloses (hemicelluloses and cellulose). Notwithstanding, some of them are considered as selective since they lead to significant lignin losses with little carbohydrate consumption. Fungal pretreatment efficiency is strain and substrate dependent, however culture conditions also play a major role in selective delignification [5].

This study focuses on fungal pretreatment for biogas production by anaerobic digestion. Anaerobic digestion is considered to be one of the most efficient ways to produce fuel and to manage waste [9,10]. During anaerobic digestion of lignocellulosic biomass, not only is cellulose converted to methane but so are hemicelluloses. In particular, methane production from lignocellulosic biomass can be predicted using the biomass content in lignin and crystalline cellulose (negative impact), amorphous holocelluloses and protein (positive impact) [11]. Cellulose crystallinity is often reduced during WRF pretreatment that positively affects anaerobic digestion. The protein content may increase due to the presence of the mycelium although variations are generally moderate [8]. Consequently, the Holocelluloses/Lignin (H/L) ratio appears to be a suitable indicator of the pretreatment efficiency for anaerobic digestion. Measurement of the S/G (synapyl/guaiacyl) ratio can also be helpful in the study of anaerobic digestibility. Indeed, the S/G ratio influences lignin degradability because G-units are generally less degradable than S-units [7].

Several techniques such as Nuclear Magnetic Resonance (NMR) or thioacidolysis are used to chemically characterize lignocellulosic biomass. They can be very expensive to run, they require users with expertise (NMR) or can be time consuming (several hours to prepare an analysable extract for thioacidolysis). Pyrolysis-Gas Chromatography–Mass Spectrometry (Py-GC–MS) has proven to be a useful tool because it hardly requires sample manipulation, while providing data on different compounds (carbohydrates, proteins, lignin. . .) [12]. Moreover, the time of analysis is short compared to other existing methods [13].

A good estimation of the main components of lignocellulose can be obtained from certain quantitative methods; however different methods tend to produce different results. This is partly due to the difficulty in isolating pure fractions (protein residues in lignin fraction, etc.) [14], and for pyrolysis, in the incomplete release of analysable fragments. Although pyrolysis is generally a semi-quantitative method, the use of internal standard remains feasible [13]. However, for the analysis of samples with a large range of lignin contents, a correlation between Klason lignin and lignin estimated by pyrolysis for wood, grass and crop residues could be established without internal standard [13,15,16]. Py-GC–MS is considered as a sensitive, rapid and reproducible technique with almost no waste generation [17].

Some compounds formed by Py-GC–MS are known to originate specifically from lignin, polysaccharides, proteins, etc. [18]. Py-GC–MS of lignocellulose was successfully applied to study relative amounts of lignin and carbohydrates, to classify lignin based on their S/G ratio, to follow chemical changes in cell walls during plant maturation or after chemical or biological delignification. Finally, this technique was used to evaluate the origin and composition of food and feed, forest litter, compost and materials from the paper industry (pulps, papers, effluents) [17].

Few studies have applied Py-GC–MS on WRF pretreated wheat straw [17,19,20] or other lignocellulosic biomasses such as corn stover [21,22] or spruce [23] but it is noteworthy that in reflecting the chemical diversity of plants, pyrolysate composition is substrate-dependent [24–26]. Nevertheless, none of these studies is linked to anaerobic digestion, except for one carried out by Liang et al. where anaerobic digestion of rice straw was evaluated as a pretreatment for Py-GC–MS [27]. To our knowledge, Py-GC–MS has not been applied to investigate the efficiency of fungal pretreatment for anaerobic digestion of lignocellulosic biomass. Therefore the application potential of this technique does not yet seem to be fully exploited. In particular, estimation of the amount of fungal biomass with Py-GC–MS would be worthwhile in order to control fungal growth during pretreatment. In addition, as fungi are easily degraded during anaerobic digestion, the amount of fungal biomass may positively affect methane production from fungal pretreated substrate. The viable and rapid measurement of fungal biomass for different strains and culture conditions is often difficult to achieve [28].

The aim of this study was to investigate the suitability of Py-GC–MS to assess the anaerobic biodegradability of wheat straw pretreated by several fungal strains (five WRF and one Brown-Rot Fungi (BRF)). Firstly, mycelium of a white-rot fungus, fungal treated and untreated straw were analysed with Py-GC–MS and their pyrolysates were described. Secondly, relationships between pyrolysis data, other characterization results and anaerobic degradability were evaluated.

2. Material and methods

2.1. Sample preparation

2.1.1. Fungal strains and straw substrate

Winter wheat straw (*Triticum aestivum*) was obtained from Vivescia (Reims, France). It was harvested in the North of France in 2012.

As previously described by Zhou et al. [28], basidiomycete strains used in this study belong to the "Centre International de Ressources Microbiennes" dedicated to filamentous fungi of biotechnological interest (CIRM-CF) and maintained at the French National Institute of Agricultural Research (INRA; Marseille, France). Efficient WRF strains [29,30] were used to pretreat straw for anaerobic digestion (Table 1). An inefficient Brown-Rot Fungi (*Gloeophyllum trabeum* BRFM 236) [30] was used for comparison.

2.1.2. Cultivation of Polyporus brumalis BRFM 985 on liquid medium

Mycelium of *Polyporus brumalis* BRFM 985 was cultivated on liquid medium (malt broth extract 20 g/L) in Roux flasks plugged with cotton wool. All materials and culture medium were autoclaved for 20 min at 120 °C. Inoculation was performed with five agar discs (5-mm diameter) of 7-day old mycelia. After 10 days at 30 °C, mycelium was harvested, washed extensively with ultrapure water and freeze-dried.

2.1.3. Fungal pretreatment

As previously described [29], fungal pretreatments were carried out in glass columns which contained 20 g total solids (TS) of autoclaved ground wheat straw (4 mm), 25 mg of glucose/g Total Solid (TS) and 2.5 mg of tartrate diammonium/g TS. Column temperatures were set to 28 °C. Airflow saturated with moisture was set to 120 mL/min, measured by a ball flowmeter. For each strain, cultures in Solid-State Fermentation (SSF) columns were triplicated and the resulting pretreated wheat straws were homogenized. Culture duration lasted three weeks for all samples. However, for two strains, BRFM 985 and BRFM 1554, a second culture duration of two weeks was tested (suffix '–2 w' added to the BRFM number for the identification of corresponding samples in Table 1). These two samples were inoculated with 130 mg (dry weight) ground mycelium whereas others received 120 mg (dry weight). At the beginning of the cultivation, straw was moistened: wet straw weight (WW) corresponded to 4.5 fold the initial TS weight for