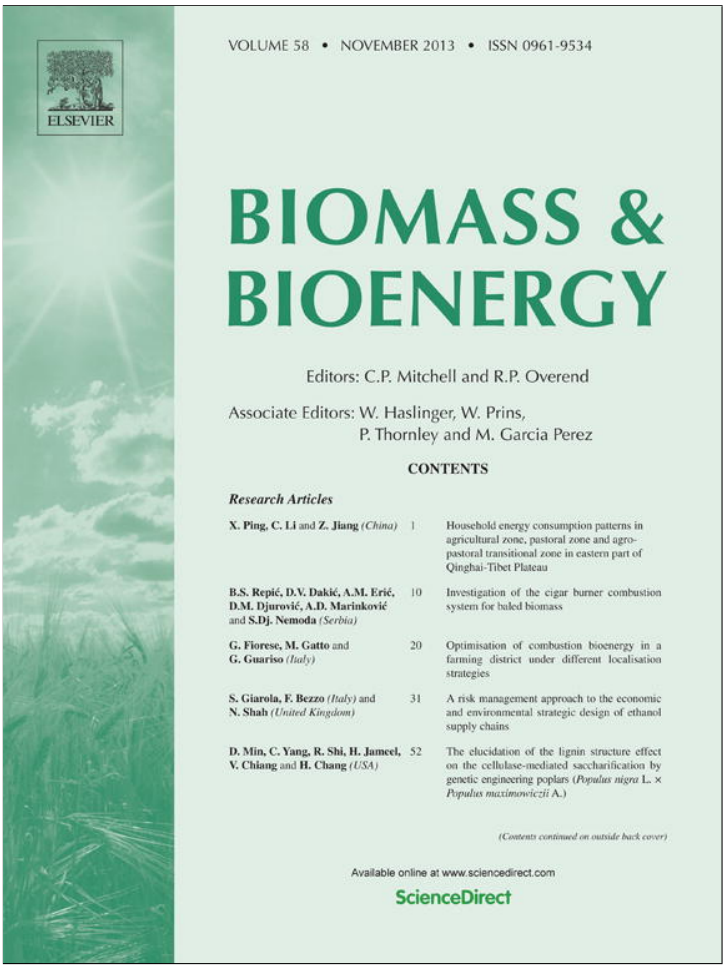

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Biodelignification of agricultural and forest wastes: Effect on anaerobic digestion

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ABSTRACT

Four lignocellulosic wastes (wood fiber, grass, corn stover and wheat straw) were treated with the ligninolytic fungus *Phanerochaete flavido-alba* to improve their anaerobic digestion. After 21 days solid substrate culture, lignin content was depleted in all materials by fungus in a range between 5 and 20%, but cellulose and hemicellulose were also biodegraded. Anaerobic biodegradability of corn stover, grass and wood fiber increased as a consequence of fungal treatment. Biogas production was enhanced only in wood fiber. Fungal delignified wood fiber produced 124 NL biogas kg^{-1} dry wood fiber with a 64% methane, after 21 days anaerobic digestion; while non-inoculated controls did not produce any biogas. Pre-digestion of agricultural wastes (corn stover, grass and wheat straw) before bio-delignification treatment failed to improve subsequent biogas production.

1. Introduction

Anaerobic digestion is a highly promising technology to convert biomass waste into methane, which may directly be used as an energy source. One of the main issues of this treatment method is the non-degradability of lignin under anaerobic conditions. In lignocellulose-rich organic wastes such as some agricultural and forest wastes, this component decreases availability of other more easily biodegradable components such as cellulose and hemicellulose because it acts as a barrier against microorganisms and their enzymes. In fact, some authors propose mathematical models based on lignin content to predict anaerobic biodegradability or methane yields for organic substrates [1,2]. Consequently, lignin limits conversion of organic carbon into methane. Thus, any attempt focused to decrease lignin content would allow an increase in methane yields.

Several efforts have to improve anaerobic biodegradation of organic wastes by means of pre-treatments aimed at modifying lignocellulose composition and structure, thus facilitating further hydrolysis of fermentable sugars. These include physical (shredding, irradiation, thermal and pressure

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shocks, etc.), chemical (hydrolysis with alkali, acids or oxidants) and biological (with fungi, actinobacteria or their enzymes) treatments [3–5]. Regardless of progress in this field, there are still several issues to be investigated, and lignin biodegradation is one of the most challenging [6,7].

Although many different types of microorganisms are capable of degrading and utilizing cellulose and hemicellulose as carbon and energy sources, a much smaller group of microorganisms, especially fungi, has evolved the ability to break down lignin. Different groups of fungi have been reported as producers of ligninolytic enzymes [8], among which white-rot fungi have received extensive attention because of their powerful production of these enzymes and their unique ability to efficiently degrade lignin to CO_2 [9]. Such an extent of degradation is due to the strong oxidative activity and low substrate specificity of their ligninolytic system, which is primarily comprised of laccase (Lac), lignin peroxidase (LiP), and manganese peroxidase (MnP) [10]. These enzymes are oxidases that require oxygen (laccase) or hydrogen peroxide (peroxidases) and they are not active under anaerobic conditions [11,12]. Although there are some evidences of hydrolysis of one of the main linkages in lignin structure, β-O-4 bonds, in lignin oligomers under anaerobic sulphate-reducing conditions [13,14], these are not optimal conditions for methane production. Moreover, it has not been demonstrated for the whole lignin molecule.

Phanerochaete chrysosporium is the most intensively studied white-rot fungus due to its ability to degrade a wide range of organic substrates [15–17]. The lignin-degrading system of *P. chrysosporium* includes LiP and MnP but no laccase has been detected so far [18]. Other *Phanerochaete* species are currently being studied for the production of lignocellulolytic enzymes [19]. *Phanerochaete flavido-alba* has been reported to produce the three ligninolytic enzymes [20,21] and to grow on lignocellulosic substrates without additional nutrient sources [22]. Consequently, this fungus is a promising microorganism for lignocellulose-rich materials pretreatment.

In this work the treatment of wood fiber and agricultural wastes with *P. flavido-alba* was studied with the aim of decreasing lignin content and make cellulose and hemicellulose more accessible to microbial attack. This pre-treatment would improve further anaerobic digestion and biogas production, thus leading to a valorization of wastes. Wood fibers, along with other lignocellulose-rich materials such as grass, corn stover and wheat straw were treated with the fungus, and the impact of this treatment on anaerobic biodegradability and biogas production was evaluated.

2. Material and methods

2.1. Lignocellulosic substrates

Four lignocellulosic materials were tested, namely wood fiber, grass of verge, corn stover and wheat straw. Wood fiber was Filtracel EFC 1000 grade of Rettenmaier (Rettenmaier & Söhne Gmbh, Germany). All samples were dried at 40 °C and agricultural wastes (grass, corn stover and wheat straw) were shredded with a mill SK 100/C (Retsch Gmbh, Germany) and sieved on a 1 mm screen.

2.2. Treatment of lignocellulosic substrates with the ligninolytic fungus

Lignocellulosic materials were treated with the ligninolytic fungus *P. flavido-alba* ATCC 12679 in solid substrate culture. For treatment, 200 g of substrate were placed in 2 L flasks and sterilized in autoclave (121 °C, 20 min). The substrates were inoculated with 240 mL of a culture of fungus on DPL-ABTS medium previously incubated at 30 °C for 10 days. DPL-ABTS medium had the following composition per Liter: 10 g glucose, 5 g peptone, 2 g yeast extract, 1 mM ABTS (2,2′-azinobis-(3-ethylbenz-thiazoline-6-sulfonic) acid) and 70 mL of Kirk mineral solution [23]. Inoculum was thoroughly mixed with substrate until a homogenous mixture having 60% moisture was obtained. Non inoculated (NI) controls were run in parallel. These were prepared in same conditions as inoculated (I) but the DPL-ABTS medium was not inoculated with the fungus. This was done to make sure that the only difference between samples was the fungus treatment. Three replicates were included for each substrate inoculated and non-inoculated. Flasks were incubated for 7 days at 30 °C, and then 100 mL of fresh DLP-ABTS medium was added in aseptic conditions in order to stimulate fungus growth and ligninolytic activity [24]. Cultures were incubated for 14 days more at 30 °C. At the end of the incubation period (21 days), the materials were treated with a water steam current (100 °C) during 20 min followed by desiccation at 105 °C to eliminate fungal viability. Treated samples (I and NI) were further subjected to anaerobic digestion.

Viable spores of fungus were analyzed at different times by counts of fungus colonies in PDA (potato-dextrose-agar) Petri dishes. Appropriate dilutions obtained from 1 g of solid samples taken aseptically were seeded in PDA plates and incubated at 30 °C for 5 days. Results are expressed as logarithm of colony forming units per gram of sample (Log CFU g^{-1}).

Samples were analyzed for total organic carbon (TOC) and lignocellulose fractions. TOC was determined in a Shimadzu TOC-VCSN equipped with solids unit SSM-5000A (Shimadzu, Tokyo, Japan). The hemicellulose, cellulose and lignin contents were determined according to Van Soest's method [25] by fiber analyzer Ankon (Ankon Technology, Macedon, NY, USA). Dry weight of substrate in flask was determined before and after fungal treatment and the total amount of fiber component was calculated for each sampling time on the basis of total substrate dry-weight in the flask. Degradation of lignin, cellulose or hemicellulose at the *n*th day was calculated by the following formula:

$$\%\ \text{Polymer degradation} = \frac{m_0 - m_n}{m_0} \times 100$$

where m_0 and m_n are the content of lignin, cellulose or hemicellulose at initial and *n*th day sampling time, respectively.

2.3. Anaerobic digestion of biodelignified substrates

The biodegradability of products in a solid state anaerobic digestion system was determined through high-rate dry anaerobic batch fermentation. The inoculum was derived from a properly operating anaerobic digester functioning with pre-

treated household waste as a sole substrate. The digester operated for a period of at least 4 months on household waste with a retention time of a maximum of 30 days and under dry (>20% solids) and thermophilic conditions (52 °C). Gas production was at least 15 mL at standard temperature and pressure (NmL) of biogas per gram of dry matter in the digester and per day on the average for at least 30 days. Before use in the anaerobic biodegradability test, the inoculum was stabilized during a short post-fermentation of 7 days to reduce the biogas production rate. This means that the concentrated inoculum was not fed but allowed to post-ferment the remains of the previously added organics. This was done to ensure that large easily biodegradable particles were degraded during this period and to reduce the background level of biogas from the inoculum itself. Inoculum was analyzed for pH, moisture content, total solids (TS), volatile solids (VS), ash, ammonium nitrogen (NH_4^+–N) and volatile fatty acids (Table 1). The pH was measured by a pH meter (CONSORT C861, OKW, Belgium) after dilution with distilled water at a ratio of 5 to 1. Total solids were determined after 24 h at 105 °C. Volatile solids were determined after 4 h at 550 °C in a muffle furnace (N17/HR, Nabertherm, Germany). Ammonium nitrogen (NH_4^+-N) was determined by spectrometer detection in Fiastar 5000 Analyser (Foss, Sweden) on aqueous extract (5/1 ratio). Volatile fatty acids were measured by gas chromatography on Stabilwax 30 m column (GC Clarus 480, Perkin Elmer, USA).

At the start of the digestion period, an amount of inoculum sufficient to prepare all test vessels was removed from the post-fermentation digester and carefully mixed. The anaerobic digestion tests were performed according to the guidelines of ASTM D5511-02 [26] and ISO 15985:2004 [27]. Each 4 L reactor was filled with 1 kg of inoculum and 15 g of reference cellulose (Merck) or test item, except for the blank reactors (no test material) and the corn stover reactors (20 g for treated corn stover and 25 g for non-treated corn stover). The tests were performed in duplicate. The reactors were incubated at a temperature of 52 °C and connected to a gas measuring device in which the produced biogas was stored in an inverted graduated cylinder to measure the total volume of biogas produced (Supplementary Fig. 1). A gas sampling point was attached to each cylinder and gas samples (5 mL syringe, Gastight® #1005, Hamilton Co., Reno, Nevada, USA) were taken during the anaerobic digestion. CH_4 and CO_2 contents in the biogas were measured using a Clarus 500 gas chromatograph (Perkin Elmer, USA) equipped with a Porapak-Q-column (12 ft × 2 mm; 60/80) and a thermal conductivity detector. The amount of CH_4 and CO_2 produced per weight unit of test item was calculated. The measured volume of gas produced was converted to the volume of gas under standard conditions for pressure and temperature. Under standard conditions, the gas law states that 22.414 NL of gas contains one mol of gas. As biogas consists of CH_4 and CO_2, each mol of biogas contains one mol of carbon (given that other components in the biogas are negligible). Using the molar mass of carbon (12 g mol^{-1}), the mass of carbon released from each reactor can be calculated. On the basis of the carbon content of the test item the degree of biodegradation was calculated as follows:

$$\%\ \text{biodegradation} = \frac{C_g(\text{test}) - C_g(\textit{blank})}{C_i} \times 100$$

with:

C_g the amount of gaseous carbon produced (in respectively the test and blank reactors)
C_i the amount of carbon added as the test item

Table 1 – Characteristics of the inoculum of the high solids anaerobic biodegradation test.

Characteristics	Value
Total solids (TS %)	17.8
Moisture content (%)	81.2
Volatile solids (VS % on TS)	49.9
Ash (% on TS)	50.1
pH	8.0
NH_4^+–N (g kg^{-1})	2.2
Volatile fatty acids (g kg^{-1})	<0.14

3. Results

3.1. *Biodelignification of fibers*

P. flavido-alba grew in all substrates tested (grass, corn stover, wheat straw and wood fiber). Colonization by the fungus mycelium was visually evident after three days incubation (Supplementary Fig. 2). In the course of treatment, spore counts were lower in wheat straw than in the other substrates. All cultures reached spore counts values around 10^5 CFU g^{-1} at the end of the incubation (21 days) (Fig. 1a). The addition of fresh DPL-ABTS medium after 7-days incubation led to an increase of fungal spore counts of 2 logarithmic units in grass and corn stover and 1 logarithmic unit in wheat straw and wood fibers. Thus, fungal population was significantly stimulated by the renewal of its medium, as indicated by an increase in fungal population higher than that obtained in the first week.

Dissimilar growth levels among the different substrates were well related to organic carbon degradation rates. Total organic carbon (TOC) was analyzed at the end of the treatment both in substrates inoculated with the fungus (I) and in non-inoculated controls (NI). TOC decrease was higher in corn stover (35%) and grass (12%) than in wood fiber (9%) or wheat straw (4%) (Table 2). Thus, carbon was less depleted in substrates of lower nutritional quality to support fungal growth, such as wheat straw, than in the other substrates.

At the end of the treatment with fungus, the substrates were heavily colonized as visually noticed by the white cover of mycelium (Supplementary Fig. 2). As a consequence of treatment, wood fiber turned from yellowish to brownish color. This color change was unexpected since lignin degradation usually causes bleaching of lignocellulosic substrates. In fact, the fungi having predominant ligninolytic activity (and less cellulolytic and hemicellulolytic), are named white-rot fungi because they turn the wood white [28]. Thus, brownish color in wood fiber could be due both to ABTS added, that turns dark after oxidation by microorganism, and to the concomitant degradation of cellulose along with lignin, whose decrease causes a brownish color. The latter was confirmed by results obtained in the analysis of lignocellulose fractions in

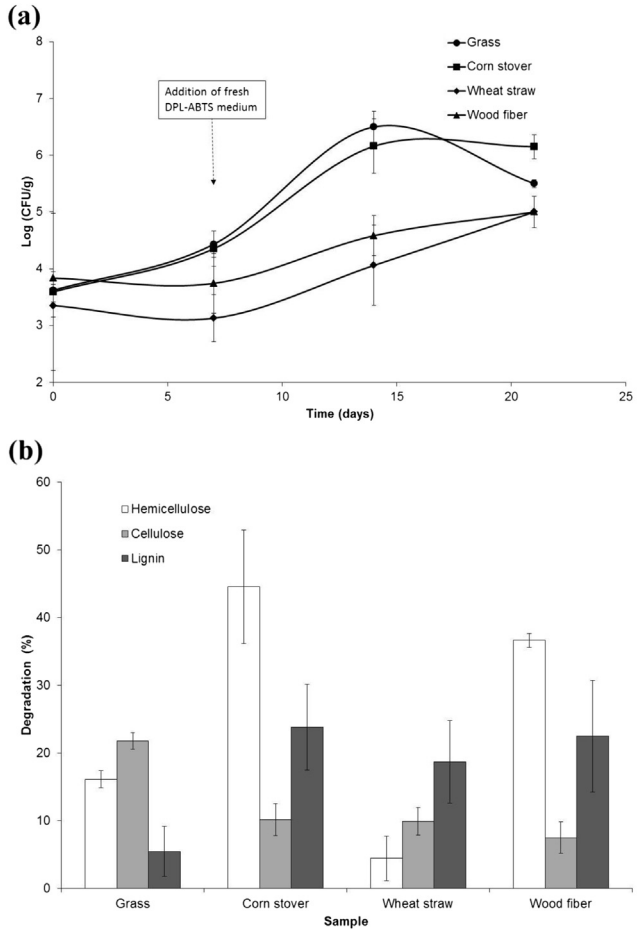

Fig. 1 – Evolution of the growth of *P. flavido-alba* on lignocellulosic substrates grass (●), corn stover (■), wheat straw (◆) and wood fiber (▲) during treatment (a); and biodegradation of hemicellulose (□), cellulose (▩) and lignin (▩) by *P. flavido-alba* after 21 days (b). Fresh DPL-ABTS medium was added after 7 days. Error bars represent standard deviation ($n = 3$).

materials (Table 2). Grass and corn stover had a similar composition with hemicellulose and cellulose contents around 25%, and lignin about 15%. Wheat straw had significantly higher cellulose and hemicellulose contents but a lignin content similar to the other two agricultural fibers. The ratio of lignocellulose components in wood fiber was quite different to the other substrates, mainly because of its higher cellulose and lignin content. Similar results have been reported for wood and agricultural fibers [29,30]. Those differences in composition may explain the dissimilar fungus growth and organic carbon decrease obtained in the substrates (Fig. 1a and Table 1). Hence, substrates with lower lignin content such as grass and corn stover have more carbon sources easier to degrade by the fungus; contrariwise, carbon fractions in wood fiber are less accessible to microorganisms because of lignin, and limit or slow down growth and organic matter degradation [31]. In the case of wheat straw, whose lignin content was comparatively low, there must be other factors that influence low growth and TOC decrease levels obtained in this substrate. This material is known to have wax and fat [32] that also limit access of fungi to carbon resources and decrease biodegradability.

Table 2 – Lignocellulose and total organic carbon (TOC) in substrates treated with *P. flavido-alba* (I) and non-inoculated controls (NI) after 21 days culture.

Substrate	TOC (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)
Grass (NI)	41.7	23.0	30.3	15.2
Grass (I)	36.8	19.3	23.7	14.4
Corn stover (NI)	25.1	25.2	25.6	16.2
Corn stover (I)	16.4	14.0	23.0	12.4
Wheat straw (NI)	43.3	34.8	48.2	12.8
Wheat straw (I)	41.6	33.3	43.4	10.4
Wood fiber (NI)	48.0	15.8	59.2	32.3
Wood fiber (I)	43.7	10.0	54.7	25.0

According to data shown in Fig. 1b, treatment of lignocellulosic substrates with *P. flavido-alba* led to a decrease in all lignocellulose fractions at different levels in each substrate. Lignin remained practically unmodified in grass but decreased a 20% in the other substrates (Fig. 1b). In absolute terms the highest decrease in lignin content was observed for the wood fibers. Cellulose was the main polymer degraded in grass but in the other substrates less than a 20% of the cellulose was depleted. Differences among substrates were more pronounced in the case of hemicellulose. This polymer was metabolized by the fungus mostly in corn stover and wood fiber (about 40% decrease), followed by grass (16%) and wheat straw (4%). Therefore, carbohydrate fractions were barely degraded in wheat straw compared to the other substrates even though lignin was degraded by the fungus at similar rate. These results support the previously stated hypothesis regarding the possible effect of factors other than lignin that contribute to the low accessibility of carbon sources to *P. flavido-alba* in wheat straw. The differences in biodegradation of lignocellulose fractions by *P. flavido-alba* among the substrates tested could be due to the diverse structure and composition of lignin which varies among vegetal species and growth phase [29]. Also, bonds between lignin and carbohydrate polymers in lignocellulose may be different [33]. These variations in lignocellulose composition, along with the fact that ligninolytic enzymes attack lignin in a random fashion [34], may cause different patterns among substrates in the amount and type of carbohydrate made accessible after lignin degradation. In this work, substrates in which the fungus degraded lignin at similar rates (wood fiber, wheat straw and corn stover), the biodegradation of carbohydrates were quite different. However, substrates in which lower lignin degradation was obtained showed the highest cellulose degradation. Although some external carbon sources (glucose, yeast extract) were added with the inoculum and fresh DPL-ABTS medium, it is obvious that *P. flavido-alba* metabolized carbon from lignocellulose during treatment. Externally added carbon sources were easily biodegradable and they were likely depleted in short time. Results obtained revealed that treatment of lignocellulosic substrates with *P. flavido-alba* in general caused a decrease in lignin content (biodelignification). Therefore, it can be concluded that the proposed method is successful for partial delignification of lignocellulosic substrates.

3.2. *Anaerobic digestion of biodelignified substrates and biogas production*

Substrates treated with *P. flavido-alba* (I) and non-inoculated controls (NI) were subjected to anaerobic digestion, and anaerobic biodegradability was analyzed. The characteristics of the inoculum used for anaerobic digestion are given in Table 1. It is recommended by the American Standard ASTM D5511-02 [26] and the international standard ISO 15985:2004 [27] that the pH is between 7.5 and 8.5, the NH_4^+ –N content between 0.5 and 2.0 g kg^{-1}, and the volatile fatty acids content < 1 g kg^{-1}. The NH_4^+ –N content of the inoculum had a value of 2.2 g kg^{-1} which is slightly higher than recommended, but still acceptable (as was also demonstrated by the good degradation of the reference item cellulose).

Fig. 2a shows the results of anaerobic biodegradability of samples tested. In general, anaerobic biodegradation was higher in the inoculated (I) samples than in the non-inoculated (NI). Anaerobic biodegradability was improved as a consequence of treatment with fungus in the case of grass, corn stover and wood fiber but did not lead to any change in this quality for wheat straw. Anaerobic biodegradation of corn stover increased in a 48% and that of grass in a 6% in comparison to biodegradation in non-inoculated materials (NI). The sharp increase in biodegradability obtained for wood fiber is remarkable. Control samples (NI) of this substrate were not degraded after 20 days digestion, while 14% degradation was obtained for samples treated with the fungus (I). It is also worth noting the high biodegradation level obtained for corn stover (I) that reached a 68% after 20 days, just a 25% lower than maximum of 95% obtained for reference material cellulose. It is important to note that this substrate had the highest hemicellulose and lignin degradation rates during treatment with fungus (Fig. 1b) and could likely have more cellulose available for anaerobic biodegradation. It can be concluded that except for wheat straw, treatment of lignocellulose materials with lignocellulolytic fungus *P. flavido-alba* increases anaerobic bioavailability of organic matter.

In spite of the good results obtained in anaerobic biodegradability of fungal treated samples, biogas production in agricultural wastes was not as well as expected (Fig. 2b). In general, biogas production was similar in non-inoculated substrates (NI) than in inoculated (I). The unique substrate in which pre-treatment with fungus led to an increase in biogas production was wood fiber. Inoculated samples of wood fiber (I) produced 124 NL biogas per kg dry wood fiber with a 61% methane, while non-inoculated controls (NI) did not produce any biogas.

The behavior obtained for agricultural wastes, good anaerobic biodegradability but poor biogas production, may be explained by two reasons. Firstly, the treatment of substrates with fungus increases the bioavailability of carbon sources because of lignin degradation, but in parallel, some nutrients (cellulose and hemicellulose) required for biogas production are depleted during biodelignification. Thus, there are carbon losses that otherwise could have been converted to biogas. Secondly, fungus may modify substrates or causes the release of compounds that affect some important phase of the biogas production process. A candidate for this is the

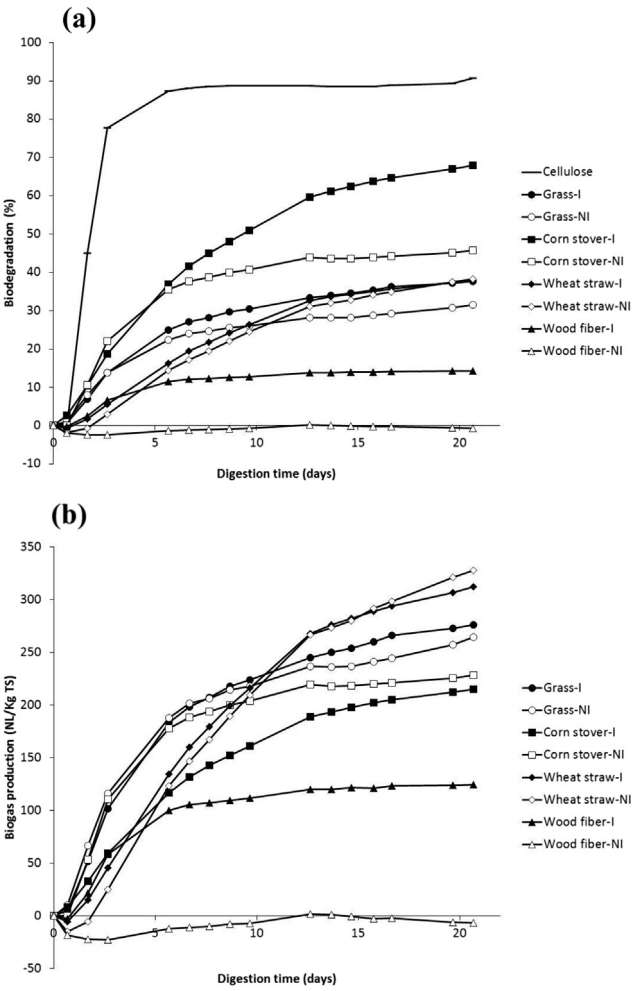

Fig. 2 – Anaerobic biodegradability (a) and biogas production (b) from reference material cellulose (▬) and from lignocellulosic substrates grass (○), corn stover (□), wheat straw (◇) and wood fiber (△) pre-treated with *P. flavido-alba* (–I) in comparison to non-inoculated controls (–NI) (black symbols).

microbial population responsible for methane production (methanogen archaea) which is known to be the most sensitive to environmental changes [35]. However, the first reason could be more plausible. In these anaerobic digestion processes the methanogen archaea seemed to be still working fine, as in the inoculated and non-inoculated reactors the CH_4 content was similar and high (>50%), indicating a good methanization process. Therefore, further studies are required to elucidate the factors involved in the failure to improve biogas production by bio-delignifying agricultural wastes. This would allow an adjustment of both pre-treatment method and anaerobic digestion in order to increase biogas production yields. Notwithstanding this, it was clear that anaerobic biodegradation, and biogas and methane production were greatly improved by biodelignification pre-treatment of wood fiber. Consequently, this can be considered a promising method to increase biogas production from wood fiber.

3.3. *Additional tests: anaerobic pretreatment-delignification-anaerobic digestion*

In an attempt to improve biogas production from agricultural wastes (i.e. corn stover, grass and wheat straw) an additional test scheme was performed. As cellulose and hemicellulose were significantly reduced in the fungus pretreatment and, as such, not available for biogas production, the idea rose to examine the effect of the treatment on the biogas production in post-digestion of digestate treated with fungus. Thus, in a first phase the agricultural wastes were anaerobically digested, degrading all reachable and anaerobically degradable organic matter. Next the digestate was treated with the fungus, breaking the lignin network which is not affected by anaerobic digestion. Finally, the pre-treated digestate was recycled to the reactor or post-digested, to produce more biogas after more organic matter is available through the breaking of the lignin network. Anaerobic digestions and treatment with fungus were performed as described in material and methods section.

Three types of digestate were selected for this test: first was derived from a reactor treating mainly grass, the second from a reactor treating mainly corn stover and the third was taken from a reactor treating mainly straw. During the treatment of the digestates with the fungus the growth of the microorganism was much lower in digested substrates than previously detected in non-digested samples. Viable spore counts were lower than 10^4 CFU g^{-1} throughout the 21 day culture period and visible mycelium was scarce. Total organic carbon was depleted by the fungus a 14% in grass digestate and less than 4% in corn stover and wheat straw digestates. Degradation of cellulose and lignin by *P. flavido-alba* was quite low but hemicellulose degradation was surprisingly high (30–60%) (Fig. 3). This result is not consistent with the low growth levels and TOC decreases (higher in grass than in corn stover or wheat straw digestate). However, the latter can be explained by the use of carbon sources other than the lignocellulose released after anaerobic digestion.

Biogas production was tested in three sample types: The first was a control (C) of digestate that had not undergone treatment with fungus and steam; the second was the substrate as described above with the fungus (I); the third was the substrate as described above without the fungus (NI). The control (C) was included to assess whether the autoclaving, shredding, and drying during the treatment had any effect. The results of these tests are shown in Fig. 4. Treated samples (NI and I) showed higher biogas production rate at the start of the test, while the untreated control samples (C) continued at a lower rate for a slightly longer period of time. However, this effect is likely due to the size reduction during the additional treatment for the NI and I series. This has been demonstrated to have an important impact on anaerobic biodegradability [36]. Fungal treatment of digestates did not lead to any improvement of biogas production as shown by the similar biogas values obtained in non-inoculated (NI) and inoculated (I) samples. Corn stover produced the highest amount of biogas followed by grass and wheat straw. From this test it can be concluded that it is not useful to treat anaerobic digestate with a ligninolytic fungus, to increase biogas production in the agricultural wastes evaluated.

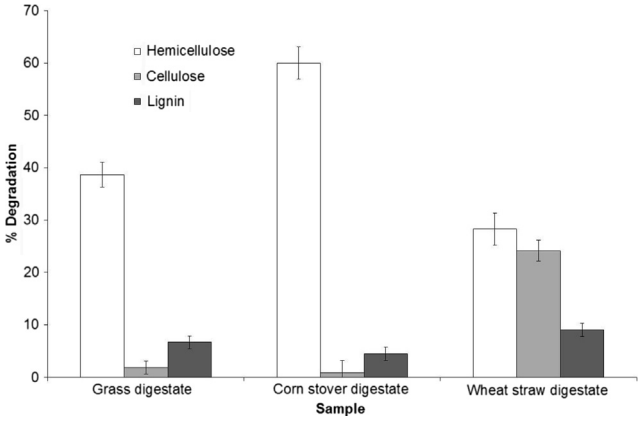

Fig. 3 – Degradation of hemicellulose (□), cellulose (■) and lignin (■) in digestate substrates due to treatment with *P. flavido-alba*. Error bars represent standard deviation ($n = 3$).

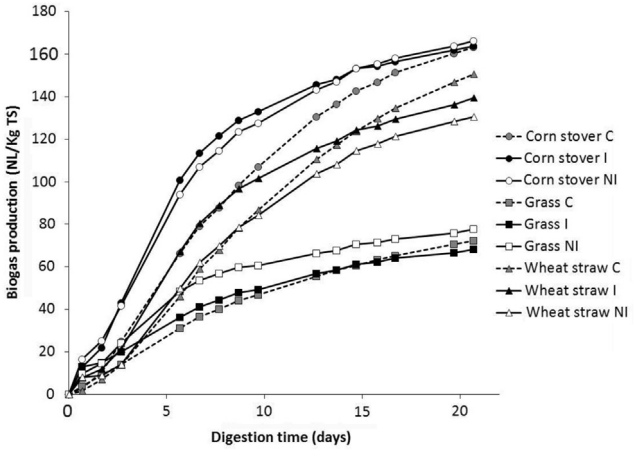

Fig. 4 – Evolution of the biogas production of the control (C) (gray symbols), inoculated (I) (black symbols) and non-inoculated (NI) (white symbols) digestates, derived from anaerobic digestion system treating mainly corn stover (○), grass (□) or wheat straw (△).

4. Conclusions

The treatment of lignocellulosic substrates with *P. flavido-alba* produced an effective biodelignification. This treatment caused a significant increase in anaerobic biodegradability and biogas production in wood fiber. Therefore biodelignification of wood fibers with *P. flavido-alba* previous to anaerobic digestion can be considered a promising pretreatment method to improve biogas production. For agricultural wastes such as grass, corn stover and wheat straw, the pre-treatment with *P. flavido-alba* had a positive effect improving anaerobic biodegradability, but none in biogas yields. Anaerobic digestion of agrowastes previous to biodelignification treatment was inefficient in order to increase biogas production. Further studies are required to adjust conditions for biogas production from agricultural wastes biodelignified with *P. flavido-alba*.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.biombioe.2013.10.021.

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