



# **Fungal pretreatment of straw for enhanced biogas yield**

**(Förbehandling av halm med svamp för ökat biogasutbyte)**

**Xinmei Feng, Maria del Pilar Castillo, Anna Schnürer**

*"Catalyzing energygas development  
for sustainable solutions"*



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Denna studie har finansierats av:  
Energimyndigheten  
Göteborg Energi AB  
E. ON. Gas Sverige AB  
LRF - Lantbrukarnas Riksförbund  
JTI - Institutet för jordbruks- och miljöteknik

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Malmö 2013

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Malmö, Sweden 2013

Martin Ragnar  
Chief Executive Officer



## Foreword

There is the need in incorporating straw as a substrate for biogas production purposes. However, the degradation of lignocellulosic materials is somewhat restricted due to the high content of lignin that binds cellulose and hemicellulose and makes them unavailable for microbial degradation. Also, the crystalline structure of cellulose affects digestibility. Consequently, low methane yields are achieved. Different methods for pre-treatment of lignocellulosic material have been explored, for example thermal, acid, alkaline and oxidative pretreatments. However, most of them require expensive specialized instruments with substantial energy requirements.

This project was developed with the idea of testing biological pretreatment methods, which can be more environmentally friendly, and can allow increasing the methane potential from lignocellulosic materials.

The work was conducted at the Department of Microbiology, Swedish University of Agricultural Sciences, Uppsala (SLU) by PhD Xinmei Feng, JTI and with the collaboration of PhD Maria del Pilar Castillo, JTI and Prof. Anna Schnürer, SLU. We had also the collaboration of Prof. Keqiang Zhang and Liang Junfeng from the biogas research group at the Agro-Environmental Protection Institute, AEPI, Tianjin, China which participated in some of our discussions and provided us with some edible fungal strains.

We thank the participation of financiers and the members of the reference group Tobias Persson, SGC; Tisse Jarlsvik, Göteborg Energi AB; Håkan A Eriksson, E.On Gas Sverige and Lars-Gunnar Johansson, LRF. Also, thanks to Geoffrey Daniel and Katarina Ihrmark, SLU for providing us with several fungal strains.



## Summary

Among lignocellulosic materials from the agricultural sector, straw is considered to have the biggest potential as a biofuel and therefore also represents a big potential for biogas production. However, the degradation of lignocellulosic materials is somewhat restricted due to the high content of lignin that binds cellulose and hemicellulose and makes them unavailable for microbial degradation. Consequently, low methane yields are achieved.

The biodegradability of the lignocellulosic material can be increased by a pre-treatment. Optimally the pre-treatment should give an increase in the formation of sugars while avoiding the degradation or loss of carbohydrates and the formation of inhibitory by-products. The treatment should also be cost-effective.

Different methods for pre-treatment of lignocellulosic material have been explored, for example thermal, acid, alkaline and oxidative pretreatments. However, they often have a high energy demand.

Biological treatment with fungi represents an alternative method for pretreatment of lignocellulosic materials that could be comparably more environmentally friendly, easier to operate and with low energy input. The fungal groups of interest for lignocellulose degradation are the wood decaying fungi, such as the white-, brown-rot and cellulose degraders.

The purpose with this work was to increase the biogas potential of straw by using a pretreatment with fungi. Straw was incubated with fungi at aerobic conditions under certain periods of time. The growth and colonization of the straw by the fungi was expected to increase the availability of the lignocellulosic structure of the straw and thus positively affect the biogas potential. In addition also, the spent lignocellulosic material from the cultivation of edible fungi was investigated. We hypothesized that also growth of edible fungi could give a more accessible material and thus give higher biogas potential compared to the substrate before fungal growth.

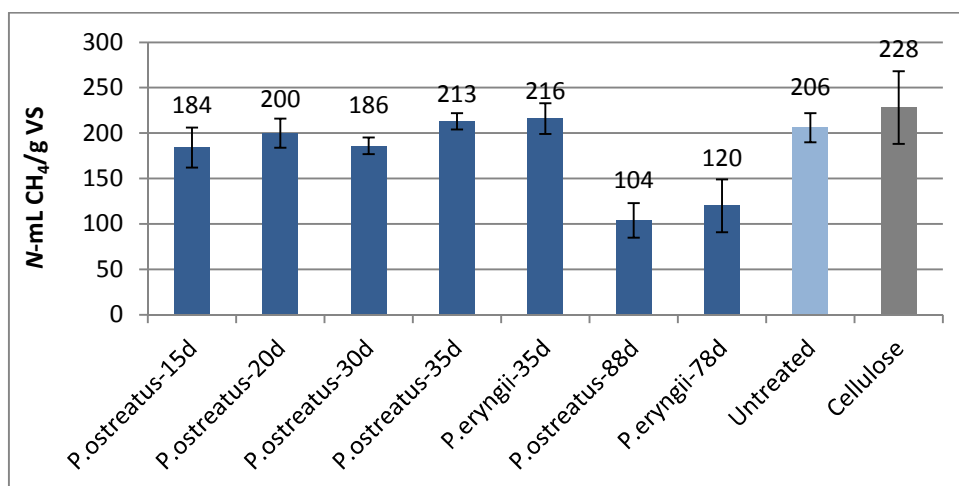


Figure i. Methane potential from fungal pre-treated straw. *Pleurotus* spp were used in this part of the study and different pretreatment times were tested. Two controls were included, one with untreated straw to calculate the effects before and after pretreatment, and one with cellulose to evaluate the inoculum activity. Longer pre-treatment times give lower methane potential.



The results showed that all investigated fungi could grow well on the straw. However, all the results from the methane potential analysis showed that fungal pre-treated straw, at the conditions tested, gave no significant higher biogas potential than untreated straw. Moreover, some carbon from the straw was lost, during the growth of the fungi under prolonged pretreating times, resulting in lower methane yields compared to non-treated straw (Fig. i).

Spent compost material from growth of edible fungi as *P. eryngii* keeps 68 % of the initial methane potential and can be an interesting biogas substrate (Fig. ii). However, the methane potential in the *A. bisporus* spent compost was too low to be of interest for the biogas industry.

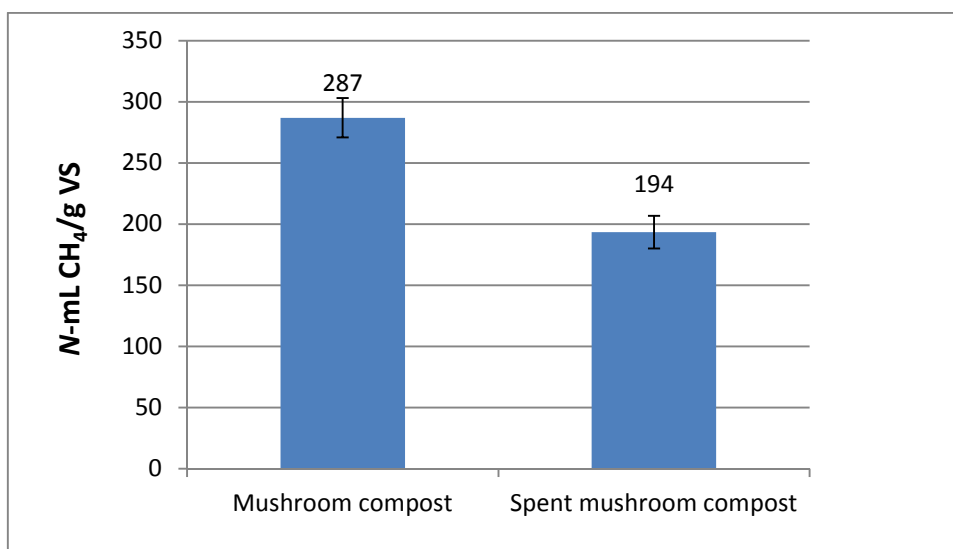


Figure ii. Methane potential from mushroom compost use for growth of edible fungi (*P. eryngii*). Mushroom compost denotes the substrate before the fungal growth and spent mushroom compost after harvest of the fungi.

In contrary to our results, some literature reports show that fungal treated straw can result in increasing methane potentials. A possible explanation to the difference in result are different degradation efficiency of the inoculums used in the methane potential tests or selection of manure as inoculums or as a material used during co-digestion of straw material. To completely resolve the impact of fungi on lignocellulosic materials and clarify the possibility of using them for pre-treatment, further studies are recommended.



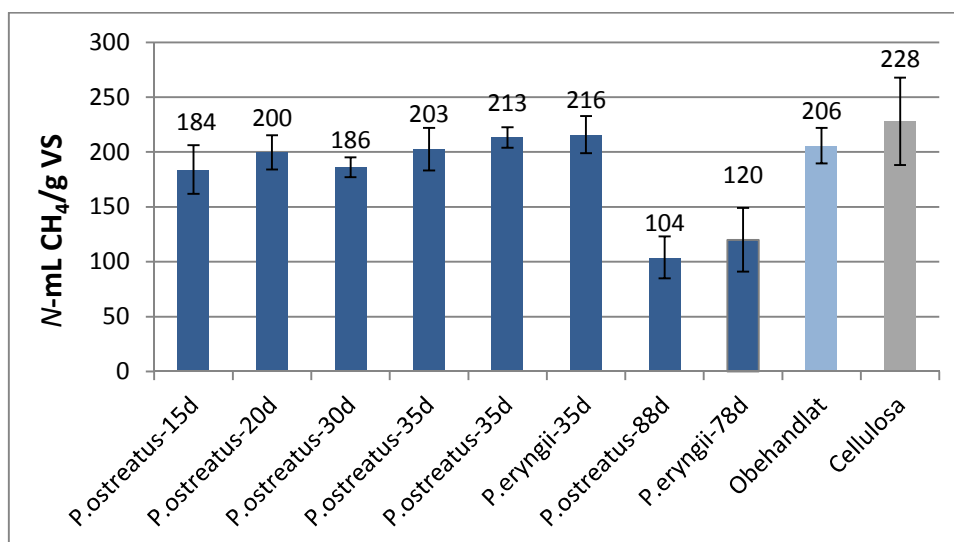
## Sammanfattning på svenska

Bland lignocellulosamaterial från jordbrukssektorn anses halm ha den största potentialen som biobränsle, inklusive för biogasproduktion. Emellertid är nedbrytning av denna typ av material något begränsad på grund av hög halt av lignin, som binder cellulosa och hemicellulosa och gör materialet otillgängliga för mikrobiell nedbrytning. Därför uppnås låga metanpotentialer.

Den biologiska nedbrytbarheten av material rikt på lignocellulosa kan ökas genom en förbehandling. Optimalt ska förbehandlingen bryta sönder den komplexa strukturen och ge en ökad halt av enkla sockerarter och samtidigt inte leda till nedbrytning eller förlust av kolhydrater eller bildning av inhiberande biprodukter. Behandlingen bör också vara kostnadseffektiv. Olika metoder för förbehandling har undersökts, t.ex. behandling med hög temperatur, syra, alkali och oxidativa ämnen. Många av dessa metoder är mycket energi krävande och kräver stora mängder kemikalier.

Biologisk behandling med svampar representerar en alternativ metod för förbehandling av lignocellulosa material som har potentialen att ha en låg energiförbrukning, vara mer miljövänlig och enklare att använda jämfört med kemiska och termiska metoder. Svampar av intresse för biologisk behandling är vit- och brunrötesvampar samt cellulosa nedbrytare, som alla har potentialen att påverka strukturen hos lignocellulosabaserade material.

Syftet med detta projekt var att öka biogaspotentialen av halm genom förbehandling med svampar. Olika svampar fick växa på halm vid aeroba förhållanden under olika tidsperioder. Tillväxt och kolonisering av svamparna på halmen förväntades ge förändringar på den komplexa strukturen hos lignocellulosa och därmed ha en positiv påverkan på biogas potentialen. I studien undersöktes också kompost material som används för odling av matsvamp. Här var förväntningen att ett förbrukat kompostmaterial, kvar efter tillväxt av matsvampen, skulle ge högre metanpotential än samma material före svamptillväxt.

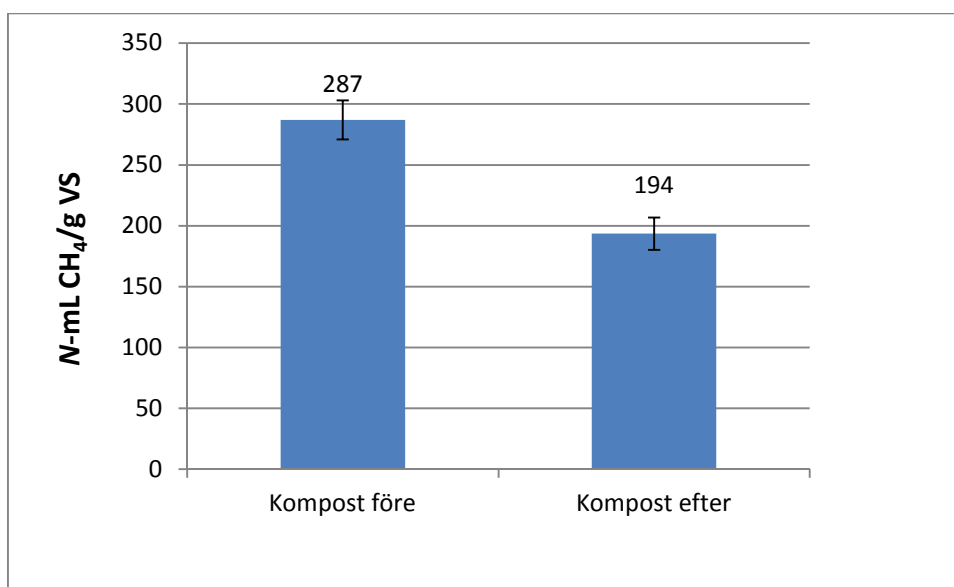


Figur iii. Metanpotentialen på halm förbehandlad med olika svampar. Två olika *Pleurotus* arter med olika förbehandlingstider utvärderades tillsammans med obehandlad halm och cellulosa som kontroller.



Resultaten visade att alla undersökta svampar växte bra på halmen. Emellertid gav förbehandlad halm, under de betingelser som testades här, inte någon signifikant högre biogaspotential jämfört med en obehandlad halm. Resultaten visade också att förbehandlingen resulterade i en förlust av kol från halmen, på grund av tillväxt av svampen (Fig. i). Denna kolavgång avspeglades i en lägre metanpotential från den behandlade halmen, speciellt efter en lång behandlingstid.

I projektet undersöktes också möjligheten att använda kompostmaterial, kvar efter produktion av matsvamp, *P. eryngii* (typ av ostronskivling) och *A. bisporus* (champinjon). Analys av metanpotentialen visade att komposten kvar efter tillväxt av *P. eryngii* motsvarade 68% av den ursprungliga metanpotentialen av materialet och därför kan vara ett intressant biogassubstrat (Fig. iv). Kompostmaterial kvar efter produktion av *A. bisporus* hade däremot för låg potential för att vara av intresse.



Figur iv. Metanpotential på kompost, före och efter produktion av matsvamp (*P. eryngii*, en typ av ostronskivling).

I motsats till våra resultat redovisar flera studier i litteraturen att halm förbehandlad med svamp ger högre metanpotentialer än obehandlad halm. En möjlig förklaring till skillnaden i resultat är att de tidigare publicerade studierna är skillnad i nedbrytningseffektivitet i ympmaterialen som använts i metanpotentialanalysen. En annan möjlig förklaring är att de tidigare studierna samtliga använt gödsel som ymp eller som samrötningsmaterial under kontinuerlig biogasproduktion från halm. För att vidare utreda effekten av svampar på halm och möjligheten att använda dessa som en förbehandlingsmetod rekommenderas ytterligare studier.



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## 1. Background

There is an increasing concern about the detrimental effects of human activities on the environment. In 1999 and 2005 the Swedish Parliament established 16 environmental quality objectives. The first objective, Reduced Climate Impact (Be-gränsad klimatpåverkan) states that, according to the UN Framework Convention on Climate Change, the concentrations of greenhouse gases in the atmosphere must be stabilized at levels which ensure that human activities do not have a harmful impact on the climate system. This goal must be achieved in such a way that biological diversity is preserved, food production is assured and other goals of sustainable development are not jeopardized ([www.miljomal.nu](http://www.miljomal.nu)).

One strategy to decrease gas emissions is the replacement of fossil fuels by biofuels. Bioenergy from renewable sources is a viable alternative and among different possible alternatives the biogas technology (anaerobic digestion) is in focus in Sweden, Europe and worldwide due to its potential to be an important tool for sustainable development of society. Theoretically, biogas can be produced from almost any organic material. Traditionally, sewage sludge, manure and food waste have been the major sources for biogas production but lately lignocellulose materials have gained increased attention. Today the biogas production is about 1.5 TWh/year but if lignocellulose materials, except forest residues, were to be included the potential can increase to ~15TWh/year (1).

Among lignocellulosic materials from the agricultural sector, straw is considered to have the biggest potential as a biofuel (1). Today, in Sweden about 100 000 tons of straw are used for energy production through incineration, but the potential has been calculated to be 1 million ton of straw/year (<http://www.bioenergiportalen.se/>) (2). Straw also represents a big potential for biogas production. However, the degradation of lignocellulosic materials is somewhat restricted due to the high content of lignin that binds cellulose and hemicellulose, making them unavailable for microbial degradation. Also, the crystalline structure of cellulose affects digestibility. Consequently, low methane yields are achieved.

The biodegradability of the lignocellulosic material can be increased by a pre-treatment, with the purpose of removing lignin, hydrolyze hemicellulose, decrease cellulose crystallinity, increase the porosity of materials and make the material more accessible for microbial and enzymatic attack (3). Optimally the pre-treatment should give an increase in the formation of sugars while avoiding the degradation or loss of carbohydrates and the formation of inhibitory by-products. The treatment should also be cost-effective (4).

Different methods for pre-treatment of lignocellulosic material have been explored, for example thermal, acid, alkaline and oxidative pretreatments (3, 5). However, most of them require expensive specialized instruments with substantial energy requirements (6). Also, toxic products as furfurals, 5-hydroxymethylfurfural (HMF), organic acids, and phenols may be formed.

Biological treatment with fungi represents an alternative method for pretreatment of lignocellulosic materials that could be more environmentally friendly, easier to operate and with low energy input. The fungal groups of interest in lignocellulose degradation are the wood decaying fungi as the white-, brown-rot and cellulose degraders. Some of these fungi produce lignin and cellulose degrading enzymes (6-8) and have been proved to disrupt the lignin-cellulose bindings in straw



(9, 10). At present, little information about fungal treatment of straw for increasing biogas potential is available in literature. Mackulak et al (11) reported an increase of 15% in biogas production when hay and leaves were pretreated with the wood-decaying fungus *Auricularia auricular-judae*. Furthermore, Bisaria et al (12, 13), Mehta et al (14) and Wati et al (15, 16) reported that the use of spent lignocellulosic material from the cultivation of the edible fungi *Pleurotus* spp. produced higher biogas yields compared to untreated substrates.

To our knowledge, the biogas potential from fungal pretreated straw or from spent lignocellulosic material from edible fungi growth has not been evaluated in Sweden.

### 1.1 Aim of the project

The aim of this project was:

- a) To evaluate the biogas potential of fungal pretreated straw
- b) To analyze the biogas potential of the spent lignocellulosic material from the cultivation of edible fungi
- c) To evaluate the importance of the inoculum on the results of the biogas potential measurement, i.e. its effect on the degradation rate of treated and untreated straw as well as cellulose.

In order to achieve these goals the following studies were performed:

- Screening of fungi: several fungal strains with capabilities of affecting the lignocellulosic structure were used for pretreatment of straw. The effect of the treatment was evaluated by measuring the biogas potential.
- Strains from the edible fungi *Pleurotus* spp. were used in the pretreatment of straw, using different pretreatment times. The effect of the treatment was evaluated by measuring methane potential.
- The methane potential of spent mushroom compost collected in Sweden and China was measured.
- The influence of the anaerobic inoculum on the methane potential of treated and untreated straw was evaluated in methane potential tests.



## 2. Materials and Methods

### 2.1 Fungal strains

Twelve fungal strains from Swedish collections (Appendix, Table A.1) were studied in this work. Commercial available edible *Pleurotus ostreatus* (ostronskivling) and *Pleurotus eryngii* (kungsmussling) (Figure 1) were also studied.

### 2.2 Anaerobic inocula

The biogas inocula (A and B), used in the measurement of biogas potential, were collected from two large-scale mesophilic biogas plants. The composition of the main feedstock to the biogas plants was: inoculum A, silage and source-separated organic household waste and B sludge from wastewater treatment plant.

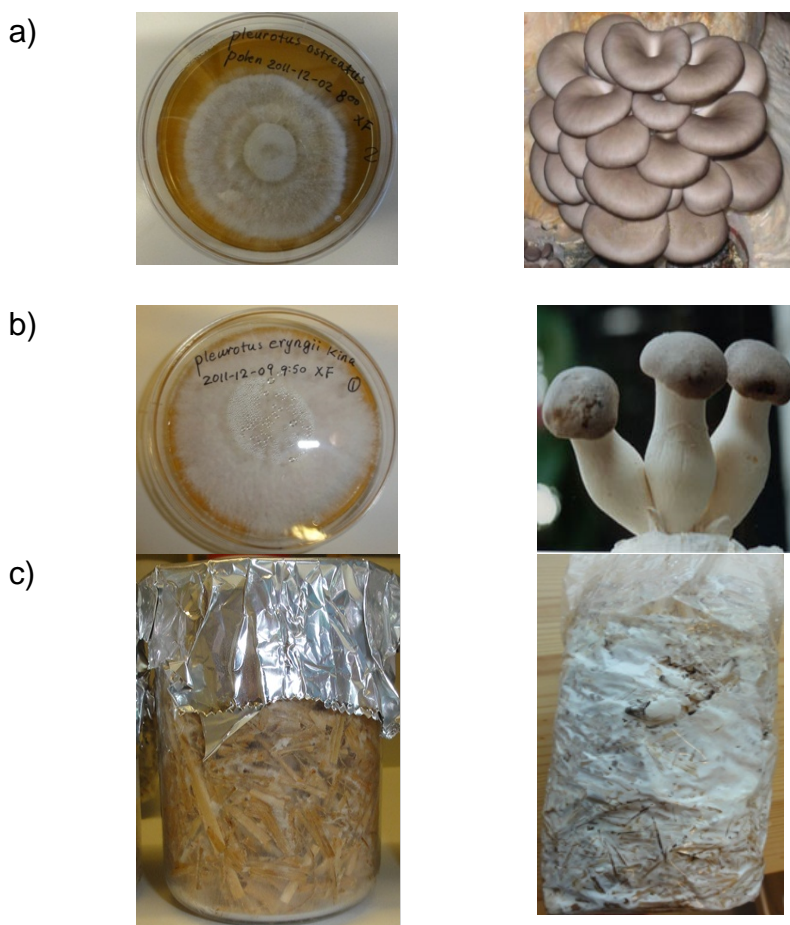


Figure 1. *Pleurotus* spp studied in this work, a) *Pleurotus ostreatus* (ostronskivling), b) *Pleurotus eryngii* (kungsmussling) and c) cultures in beakers and plastic bags.

### 2.3 Straw

Wheat straw was kindly supplied by Maria Erikson's farm at Härkeberga, Enköpings. The straw was chopped (scissors) and sieved. The fraction between 2 and 4 mm was used in the experiments.



## 2.4 Spent mushroom compost

The methane potential from spent mushroom compost produced in Sweden and China was determined. Three Swedish mushroom farms (SV1, SV2 and SV3) provided spent mushroom compost from the cultivation of *Agaricus bisporus* (champinjoner). Our Chinese colleagues provided spent material from *P. eryngii* cultivation. As a control, the substrates used prior to the fungal cultivation were also analysed in the methane potential measurements. The substrate for *Agaricus bisporus* cultivation consisted of a mixture of wheat straw, peat, lime, chicken- and horse manure. The substrate used in *P. eryngii* cultivation consisted of a mixture of sawdust, maize flour and wheat bran.

## 2.5 Pretreatment - Culture conditions

The pretreatment was performed by incubating the straw with fungi at aerobic conditions under different periods of time. The growth and colonization of the straw by the fungi was expected to give structural changes on the lignocellulosic structure and affect the biogas potential.

The chopped straw was wetted with water (30 % TS final). Depending on the trial, the wet straw, 1.8g, 30g or 160 g was weighed into 250 mL bottles, 250 mL beakers or plastic bags, respectively, and autoclaved for 30 min at 120°C. After cooling, the straw was inoculated by adding agar-mycelium plugs.

For agar plugs inoculation, fungi were grown on MEA (OXOID) plates at 25°C for around 4 to 8 days and thereafter 4 agar plugs (8 mm diameter) were removed and placed on the top of the autoclaved straw.

After inoculation the straw samples were incubated aerobically at 25°C for several days depending on the trial.

The total solids (TS) and volatile solids (VS) of the straw material (Standard Method SS 028113) were measured before and after the aerobic incubation period.

## 2.6 Biochemical methane potential - BMP

The biogas potential (18, 19) was measured in 250 mL bottles with the inoculum A in all trials except for the one in which the importance of the inoculum on biogas yield was tested. In that particular trial inoculum B was also used. The bottles were filled with a liquid volume of 175 ml (inoculum + substrate + water). The organic loading was 3.5 g VS/ L of the respective substrate (treated straw including fungal mycelia or untreated straw) and 7.0 g VS/L of inoculum. The bottles were placed on a rotary table (130 rpm) in a thermostatic room at 37 °C. The experiments were performed with three replicates. In each experiment, three replicates with only inoculum to deduct its contribution to the formation of biogas were included. Several additional controls were included in each trial and they are explained in the respective section.

Gas production was calculated by measuring the pressure in the bottles using a digital pressure gauge (GMH 3110) equipped with a pressure sensor (GMSD 2BR, -1000 to 2000 mbar). The pressure was then converted to the normal volume of gas (1 atm, 0 ° C). Gas samples were taken and analyzed on a gas chromatograph (PerkinElmer Arnel, Clarus 500; column: 7 " HayeSep N 60/80, 1/8 "SF; FID Detector 250 ° C, carrier gas: helium, flow rate 31 mL/min, injector temperature: 60 °C; injection using Headspace sampler Turbo Matrix 110). The incubation period



varied depending on the trial. Methane production was determined as the Normal-mL CH<sub>4</sub>/g VS. The maximum methane production rate (N-mL CH<sub>4</sub>/g VS/d) was determined by calculating the slope of the curves between days 1 to 10 except for sample Cellulose-A that was calculated between days 7 and 14 after the acceleration phase.

## 2.7 Biogas potential from fungal pretreated straw - Screening of fungi

Straw was pretreated with 12 fungal strains (Appendix, Table A.1) to test their ability to affect the lignocellulose structure and increase the biogas potential. The straw (1.8 g) was weighed (in 250 mL bottles), wetted, sterilized, inoculated with fungi by adding agar-mycelium plugs (see section 2.5) and incubated at 25°C for 9 days according to previous results from our Chinese partners.

At the end of the incubation period, inoculum from a biogas plant (A) was added directly to the bottles and the biogas potential measured during approximately 50 days. Controls with untreated straw and untreated straw with agar plugs without fungi were also run.

## 2.8 Methane potential from straw pretreated with *Pleurotus* spp. – effect of fungal strain and pretreatment time

Straw was pretreated with *Pleurotus ostreatus* and *P. eryngii*. The straw was weighed in 250 mL beakers (30 g) or plastic bags (160 g) size 28,5x8,0x6,5 cm, wetted, sterilized (120 °C, 20 min), inoculated with fungi by adding agar-mycelium plugs (see section 2.5) and incubated at 25 °C. Straw inoculated with *P. ostreatus* was incubated for 15, 20, 30, 35 and 88 days and straw inoculated with *P. eryngii* for 34 and 78 days. Thereafter, portions (3.5 g VS) of the pretreated straw (straw with fungal mycelia) were taken for further measurement of methane potential (see section 2.6). A control with untreated straw was also run. To evaluate the inoculum activity a control with cellulose was also included. The methane potential was measured during 53 days.

## 2.9 Methane potential from spent mushroom compost

The spent mushroom compost from Sweden and China was weighed in 250 mL bottles together with inoculum from the biogas plant A and water (see section 2.6). The organic loading of the respective compost was 3.5 g VS/L per bottle. The methane potential was measured during 27 days. Controls consisting on mushroom compost before fungal inoculation/growth were also run. To evaluate the inoculum activity a control with cellulose (C6663 Sigma cellulose fibers, long) was included.

## 2.10 Methane potential from fungal pretreated straw – effect of the anaerobic inoculum

The biogas potential of straw pretreated for 40 days with *P. ostreatus* was measured with two inocula, A and B. The methane potential (see section 2.6) was measured during 65 days. To evaluate the inoculum activity a control with cellulose was included.



### 3. Results

#### 3.1 Biogas potential from fungal pretreated straw - Screening of fungi

A summary of the results from the biogas potential measurements of the fungal pretreated straw, as well as the percentage of the difference in respect to the control (sterile straw with no addition of agar plugs = Control 2) are shown in Table 1. In addition results are also given for a second control (Control 1) i.e. sterile straw with added agar plugs in the same amount as used for inoculation of the fungi.

The results showed significant differences between the two controls, i.e. higher biogas production was observed in Control 1 compared to Control 2. This suggests that the nutrient in the agar plugs contributed to production of biogas. Furthermore, the biogas potential of some of the fungal pretreated straw samples showed significant differences compared to sterile straw (Control 2) but no difference with Control 1 (sterile straw plus agar plugs). These results suggest that a) the extra biogas produced compared to sterile straw alone may be coming from the agar plugs solely and not due to an increased availability caused by fungal pretreatment, b) the biogas potential in the pretreated straw had a tendency to produce less biogas than Control 1 (untreated straw plus agar plugs) suggesting that part of the carbon is lost during fungal growth.

*Table 1. Biogas potential (N-mL biogas/g VS) at 9, 15 and 25 days of fungal pre-treated straw*

		N-mL biogas/g VS			% of difference with respect to Control 2		
		9d	15d	25d	9d	15d	25d
A	<i>A. niger</i>	241 ± 2	358 ± 4	476 ± 12	4.2	-1.6	-2.5
B	<i>T. viride</i>	289 ± 7	409 ± 6	533 ± 14	24.7	12.6	9.1
C	<i>A. discolor</i>	211 ± 24	326 ± 17	454 ± 17	-9.2	-10.5	-7.2
D	<i>T. reesei</i>	245 ± 13	389 ± 18	490 ± 13	5.6	7.0	0.3
E	<i>P. chrysosporium</i>	245 ± 6	412 ± 11	533 ± 7	5.5	13.2	9.0
F	<i>T. versicolor</i>	211 ± 8	357 ± 14	465 ± 8	-9.0	-1.9	-4.8
G	<i>C. subvermispora</i>	216 ± 4	347 ± 8	456 ± 19	-6.6	-4.6	-6.8
H	<i>G. trabeum</i>	254 ± 6	411 ± 11	546 ± 18	9.4	12.9	11.6
I	<i>T. koningii</i>	245 ± 20	389 ± 29	490 ± 38	5.6	7.0	0.3
K	<i>P. chrysosporium</i>	245 ± 13	412 ± 16	533 ± 14	5.5	13.2	9.0
K	<i>T. versicolor</i>	211 ± 19	357 ± 16	465 ± 5	-9.0	-1.9	-4.8
L	<i>P. betulinus</i>	216 ± 14	347 ± 27	456 ± 26	-6.6	-4.6	-6.8
Control 1	Sterile straw+agar plug	282 ± 32	414 ± 9	535 ± 12	21.8	13.9	9.4
Control 2	Sterile straw	232 ± 24	364 ± 12	489 ± 23	0	0	0



### 3.2 Methane potential from straw pretreated with *Pleurotus* spp. – effect of fungal strain and pretreatment time

Good colonization of the straw by the *Pleurotus* spp was observed. The fungal mycelium covered all straw from top to bottom after approximately two weeks (Figure 1). The fungal pretreated straw was harvested (after 15, 20, 30, 35 and 88 days for *P. ostreatus* and after 34 and 78 days for *P. eryngii*) and the methane potential was measured (Table 2). The TS and VS of the straw before and after the fungal pretreatment were measured (Appendix, Table A.2).

The results showed no significant differences in methane potential for straw pretreated (35 and 34 days, respectively) with *P. ostreatus* ( $279 \pm 9$  N-mL CH<sub>4</sub>/g VS) with straw pretreated with *P. eryngii* ( $284 \pm 12$  N-mL CH<sub>4</sub>/g VS) (Table 2). Furthermore, no significant differences in methane potential were obtained for treated straw compared to untreated straw, when the pretreatment times were lower than 35 days. However, when the straw was pretreated for more than 78 days the methane potential decreased and become significant lower than the potential for the untreated straw. Also, straw treated with *P. ostreatus* showed lower methane potential than the *P. eryngii* treated straw. The control with cellulose produced 332 N-mL CH<sub>4</sub>/g which shows good activity of the inoculum.

*Table 2. Methane potential (at 8, 15, 24 and >60 days) of fungal pretreated straw. The straw was pretreated for 15, 20, 30, 35 and 88 days with *P. ostreatus* and 34 and 88 days with *P. eryngii*.*

Straw and pre-treatment time	N-mL CH <sub>4</sub> /g VS			
	8d	15d	24d	> 60d
<i>P.ostreatus</i> -15d	22 ± 6	93 ± 24	184 ± 22	260 ± 16
<i>P.ostreatus</i> -20d	24 ± 3	98 ± 15	200 ± 16	265 ± 18
<i>P.ostreatus</i> -30d	22 ± 5	86 ± 14	186 ± 9	255 ± 16
<i>P.ostreatus</i> -35d	38 ± 7	121 ± 19	213 ± 9	279 ± 9
<i>P.eryngii</i> -34d	42 ± 6	116 ± 19	216 ± 17	284 ± 12
<i>P.ostreatus</i> -88d	24 ± 6	52 ± 12	104 ± 19	237 ± 7
<i>P.eryngii</i> -78d	29 ± 1	59 ± 6	120 ± 29	274 ± 12
Untreated straw	34 ± 5	103 ± 14	206 ± 16	287 ± 16
Cellulose control	35 ± 3	109 ± 15	228 ± 40	332 ± 15

### 3.3 Methane potential from spent mushroom compost

Mushroom compost before and after the cultivation/harvesting of the fruit bodies was tested for their methane potential. Compost from the cultivation of *P. eryngii* showed 3 to 4-fold higher methane potential than the respective *A. bisporus* substrate (Table 3) and this was likely due to the higher content of organic matter in the *P. eryngii* compost. Table A.2 in the Appendix shows that the VS/TS ratio for *P. eryngii* compost was 91% compared to 66% for *A. bisporus* compost.



Table 3. Methane potential (N-mL CH<sub>4</sub>/g VS) at different days of compost material before and after cultivation with *P. eryngii* (CH1) or *A. bisporus* (SV).

	N-mL CH <sub>4</sub> /g VS			
	10d	16d	26d	47d
CH1-before <i>P.eryngii</i>	67 ± 15	147 ± 22	194 ± 37	287 ± 16
CH1-after <i>P.eryngii</i>	50 ± 2	105 ± 4	145 ± 23	194 ± 13
SV1-before <i>A. bisporus</i>			67 ± 4	
SV1-after <i>A. bisporus</i>			53 ± 8	
SV2-before <i>A. bisporus</i>			52 ± 18	
SV2-after <i>A. bisporus</i>			52 ± 4	
SV3-before <i>A. bisporus</i>			62 ± 3	
SV3-after <i>A. bisporus</i>			38 ± 7	
Untreated straw	15 ± 2	158 ± 12	220 ± 33	
Cellulose control	98 ± 14	176 ± 33	276 ± 14	347 ± 11

The methane potential (at 47 days) of the *P. eryngii* spent compost material was 194 ± 13 N-mL CH<sub>4</sub>/g VS and the potential in the original substrate (before fungal growth) was 287 ± 16 N-mL CH<sub>4</sub>/g VS (Table 3).

The methane potential in the *A. bisporus* compost before and after the fungal growth showed no significant differences and the levels were low (< 70 N-mL CH<sub>4</sub>/g VS).

### 3.4 Methane potential from fungal pretreated straw – importance of the anaerobic inoculum

From a previous work, significant differences in biogas production were found when a lignocellulosic substrate was digested with inocula from two different biogas plants. Thus, to investigate if the low effect of the fungal pre-treatment was due to choice of inoculum, an additional inoculum was used in one test series.

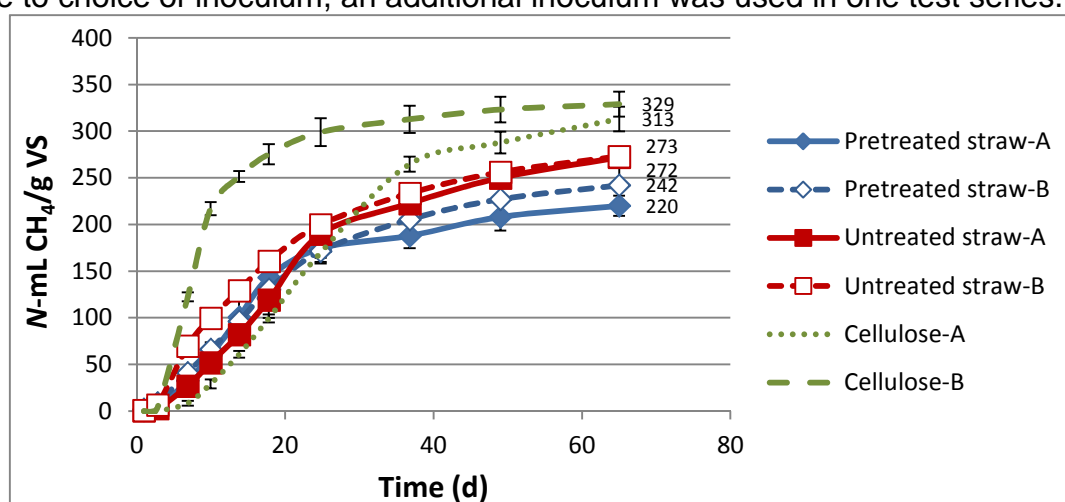


Figure 2. Accumulated methane production from fungal pretreated-, untreated straw and cellulose measured with two different inocula, A and B.



The methane potential from pretreated-, untreated-straw and cellulose was measured using inocula from biogas plants A and B (Fig. 2). The results showed no significant differences between the two inocula (after 65 days of incubation). Pretreated straw had a potential of  $220 \pm 11$  N-mL CH<sub>4</sub>/g VS with inoculum A and  $242 \pm 21$  N-mL CH<sub>4</sub>/g VS with inoculum B. The methane potential of the untreated straw was 272 N-mL CH<sub>4</sub>/g VS with inoculum A and 273 N-mL CH<sub>4</sub>/g VS with inoculum B. Cellulose had a methane potential of 313 and 329 N-mL CH<sub>4</sub>/g VS with inoculum A and B, respectively (Fig. 2).

Even though there was no significant difference in methane potential, the degradation rates obtained with the two inocula showed interesting differences (Table 4).

*Table 4. Methane production rate (N-mL CH<sub>4</sub>/g VS/d) from pretreated-, untreated-straw and cellulose incubated with inocula A and B.*

	Rate
	N-mL CH <sub>4</sub> /g VS/d
Pretreated straw-A	7.3
Pretreated straw-B	8.0
Untreated straw-A	6.8
Untreated straw-B	13.1
Cellulose-A	7.6
Cellulose-B	29.7

With inoculum B (29.7 N-mL CH<sub>4</sub>/g VS/d) an obvious higher degradation rate for cellulose was obtained compared to the results using inoculum A (7.6 N-mL CH<sub>4</sub>/g VS/d). The same trend, but not as dramatic as in cellulose, was observed with untreated straw, i.e. inoculum B (13.1 N-mL CH<sub>4</sub>/g VS/d) gave a higher degradation rate of straw than inoculum A (6.8 N-mL CH<sub>4</sub>/g VS/d). The degradation rate of the treated straw in inoculum A (7.3 N-mL CH<sub>4</sub>/g VS/d) was similar to the rate obtained with inoculum B (8.0 N-mL CH<sub>4</sub>/g VS/d).



#### 4. Discussion

Many filamentous fungi have been shown to effectively degrade various types of lignocellulosic substrates by production of lignin, cellulose or hemicellulose degrading enzymes. (11). Therefore, the purpose with this work was to increase the biogas potential of straw by a pretreatment with fungi. The pretreatment was performed by incubating the straw with fungi at aerobic conditions under a certain period of time. The growth and colonization of the straw by the fungi was expected to give structural changes on the lignocellulosic structure and affect positively the biogas potential.

In the first part of the studies several ascomycetes and basidiomycetes (white-rot and brown-rot fungi) were used for the pretreatment (Table 1 and Appendix, Table A.1). These two groups of fungi were studied because of their ability to degrade the polymers of lignocellulose material. Some ascomycetes are known to degrade cellulose and hemicellulose but have no ability to degrade lignin. Brown-rot fungi are known to degrade cellulose, hemicellulose and extensively attack and repolymerize lignin. White rot fungi can simultaneously degrade cellulose, hemicellulose and lignin at equal rates (17-19). Our results showed that despite the different enzyme pools of the studied fungal groups, none of the fungi increased the biogas potential of straw at the conditions tested in this work. The only clear pattern that was observed was related to the colonization of the straw. The ascomycetes and the white-rot showed a better and faster straw colonization than the brown-rot fungi. The results also showed that, even if not significant, a tendency of a lower biogas potential of straw in the presence of the fungi, suggesting that some carbon can be lost.

Further studies were performed with two *Pleurotus* species which are known to be highly specific lignin degraders and therefore may give less carbon (cellulose) losses during fungal growth (17). The pretreatment with *Pleurotus* spp showed no significant differences in methane potential between treated and untreated straw, when the straw was pretreated for up to 35 days. Longer pretreatment periods resulted in significantly lower methane potential compared to the untreated straw. Also, the losses were higher when *P. ostreatus* was used compared to *P. eryngii*. An explanation for these results is that part of the carbon may have been lost as carbon dioxide during fungal growth and that *P. ostreatus* grows with higher efficiency. These results imply that *Pleurotus* spp may not be as lignin selective as they were traditionally thought and that they may attack also cellulose (18).

An interesting observation was that the methane potential of the *P. eryngii* spent compost material was  $194 \pm 13$  N-mL CH<sub>4</sub>/g VS while the potential in the original substrate was  $287 \pm 16$  N-mL CH<sub>4</sub>/g VS, before fungal growth. This means that 32 % of the carbon available for methane production is lost during fungal growth and that 68 % of the methane potential still remains in the spent mushroom compost. The methane potential in the *A. bisporus* compost before and after the fungal growth showed no significant differences and the levels were too low (< 70 N-mL CH<sub>4</sub>/g VS) to be of interest for the biogas industry.

Significant differences in the methane production rate were observed when using two different inocula. Inoculum B had a 3.9-fold higher cellulose degradation rate than inoculum A (Table 4) suggesting higher levels cellulose degrading biomass and/or presence of high rate enzymes in inoculum B compared to A. The



degradation rate of untreated straw was 1.9-fold higher with inoculum B compared to A while no differences in the degradation rate were observed in the treated straw between inoculum A and B. The results suggest that lower levels of cellulose may have been available in the treated straw compared to the untreated one. That together with the fact that a tendency of lower methane potential (approx 15%) was observed in the treated straw compared to the untreated suggests that carbon was lost during the fungal pretreatment.

Our results are not in agreement with previous reports in the literature. Mackulak et al (11) reported an increase of 15% of the biogas production when hay and leaves were pretreated with the wood-decaying fungus *Auricularia auricula-judae*. Bisaria et al (12), Mehta et al (14) and Wati et al (15, 16) reported that the use of spent lignocellulosic material from the cultivation of the edible fungi *Pleurotus* spp. produced higher biogas yields compared to untreated substrates. Also, pre-treatment with *Pleurotus* spp. have been shown to increase biogas production from paddy or wheat straw by 30% (13, 20), from rice straw and *Cymopsis tetragonoloba* by 50% (12).

Possibly the difference in results could be explained by differences in the experimental set-up. Bisaria et al (12, 13) performed the studies in batch reactors using treated and untreated straw mixed with digested cattle manure as inoculum. Mehta et al (14) and Wati et al (15, 16) performed batch tests with treated and untreated straw in co-digestion with fresh cattle manure and with an unspecified inoculum. The studies by Mackulak et al (11) were performed with digested sludge from a waste water treatment plant in continuous reactors. As shown in the experiment including different inocula different results can be obtained. We did not see any difference in potential but clearly a difference in rate. Thus, both the inoculum and the incubation time of the batch test will have an impact on the results. Possibly the use of cow manure as an inoculum or co-digestion material may explain the contradictory results. Co-digestion with manure, use of digested manure as inoculum or continuous digestion processes were not part of our study. We used inoculum A from a plant treating silage and source-separated organic household waste and inoculum B from a wastewater treatment plant. The question that arises is: are there some special properties, composition, microbial communities in digested manure which make it more optimal for digestion of straw?

Furthermore, in continuous processes the continuous addition of the substrate will promote changes in the catabolic characteristics of the microflora in a more pronounced way than in a batch cultivation system. The microflora will adapt to the substrate and the biomass of the microorganisms using added material will increase and this could have a positive effect on the degradation rate of the substrate. This may explain the positive results obtained by Mackulak et al where treated hay and leaves produced 15% more biogas than the untreated substrates.



## 5. Conclusions

Different fungi and incubation conditions were tested in this study. All the results showed that straw pretreated with fungi, at the conditions tested here, gave no significant higher biogas potential than untreated straw. Moreover, some carbon from the straw may be lost as carbon dioxide during the growth of the fungi under prolonged pretreating times.

The literature reports that fungal treated straw can give higher methane potentials. However, the conditions reported in the literature were not the same as the ones used in our study. A possible reason could be the use of cow manure, either as inoculum or as a co-substrate during continuous digestion, conditions not studied in this project.

Compost material from growth of edible fungi as *P.eryngii* loses 32 % of the carbon available for methane production during fungal growth and that 68 % of the methane potential still remains in the spent mushroom compost. The methane potential in the *A. bisporus* compost before and after was too low to be of interest for the biogas industry.

Further studies are recommended. Use of digested manure and continuous digestion systems might be more suitable for observing the effects of the fungal pretreatment.



## 6. References

1. Linné, M. *et al.*, "Den svenska biogaspotentialen från inhemska restprodukter" Avfall Sverige, Svenska Biogasföreningen, Svenska Gasföreningen, Svenskt Vatten, (2008).
2. Nilsson, D., Bernesson, S., Halm som bränsle - Del 1: Tillgångar och skördetidpunkter. *SLU Report 011*, (2009).
3. Taherzadeh, M.J., Karimi, K., Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review. *International Journal of Molecular Sciences* **9**, 1621 (2008).
4. Kim, S.B., Lee, Y.Y., Diffusion of sulfuric acid within lignocellulosic biomass particles and its impact on dilute-acid pretreatment. *Bioresour. Technol.* **83**, 165 (2002).
5. Zhao, L. *et al.*, *Research progress in biogas production by anaerobic fermentation of straw-kind lignocellulosic material*. 2012 World Automation Congress (New York, 2012).
6. Taniguchi, M. *et al.*, Evaluation of pretreatment with *Pleurotus ostreatus* for enzymatic hydrolysis of rice straw. *Journal of Bioscience and Bioengineering* **100**, 637 (2005).
7. Bower, S., Wickramasinghe, R., Nagle, N.J., Schell, D.J., Modeling sucrose hydrolysis in dilute sulfuric acid solutions at pretreatment conditions for lignocellulosic biomass. *Bioresour. Technol.* **99**, 7354 (2008).
8. Herpoel-Gimbert, I. *et al.*, Comparative secretome analyses of two *Trichoderma reesei* RUT-C30 and CL847 hypersecretory strains. *Biotechnol. Biofuels* **1**, 18 (2008).
9. Li, L.-H., Ma, L.-L., Yuan, Z.-H., Liu, X.-F., Liao, C.-P., Study on anerobic digestion of strawstalk. *Journal of Agro-environment Science (China)* **26**, 335 (2007).
10. Yang, Y.-N., Chen, Y.-S., Methane production from anaerobic fermentation of straw enhanced by biological pretreatment with white-rot fungi. *Journal of Agro-environment Science (China)* **26**, 1968 (2007).
11. Mackulak, T., Prousek, J., Svorc, L., Drtil, M., Increase of biogas production from pretreated hay and leaves using wood-rotting fungi. *Chem. Pap.* **66**, 649 (2012).
12. Bisaria, R., Madan, M., Mukhopadhyay, S.N., Production of biogas from residues from mushroom cultivation. *Biotechnology Letters* **5**, 811 (1983).
13. Bisaria, R., Vasudevan, P., Bisaria, V.S., Utilization of spent agro-residues from mushroom cultivation for biogas production. *Appl. Microbiol. Biotechnol.* **33**, 607 (1990).
14. Mehta, V., Gupta, J.K., Kaushal, S.C., Cultivation of *Pleurotus-florida* mushroom on rice straw and biogas production from the spent straw. *World Journal of Microbiology & Biotechnology* **6**, 366 (1990).
15. Wati, L., Kumari, S., Diwan, P., Composting of spent oyster mushroom substrate using biogas plant slurry. *Environment and Ecology* **29**, 846 (2011).
16. Wati, L., Kundu, B.S., Singh, S., Kumari, S., Spent oyster mushroom substrate as an alternate for biogas production. *Mushroom Research* **15**, 153 (2006).
17. Liers, C., Arnstadt, T., Ullrich, R., Hofrichter, M., Patterns of lignin degradation and oxidative enzyme secretion by different wood- and litter-colonizing



- basidiomycetes and ascomycetes grown on beech-wood. *FEMS Microbiol. Ecol.* **78**, 91 (2011).
18. Arantes, V., Jellison, J., Goodell, B., Peculiarities of brown-rot fungi and biochemical Fenton reaction with regard to their potential as a model for bioprocessing biomass. *Appl. Microbiol. Biotechnol.* **94**, 323 (2012).
  19. Arantes, V. *et al.*, Fungal attack on lignin and cellulose: elucidation of brown- and white-rot mechanisms comparing biomimetic and in-vivo degradation patterns. *41st Annual Meeting of the International Research Group on Wood Protection, Biarritz, France, 9-13 May 2010*, IRG (2010).
  20. Müller, H.W., Trösch, W., Screening of white-rot fungi for biological pretreatment of wheat straw for biogas production. *Appl. Microbiol. Biotechnol.* **24**, 180 (1986).



## 7. Appendix

Table A.1. Isolates used during the screening study

	Code	Name	Supplier		Degrades
A	J175	<i>Aspergillus niger</i>	SLU	Ascomycota	Cellulose, hemicellulose
B	J644	<i>Trichoderma viride</i>	SLU	Ascomycota	Cellulose, hemicellulose
C		<i>Anthracoxyllum discolor</i>	UFRO, Chile	<i>Basidiomycota white-rot</i>	Cellulose, hemicellulose, lignin
D	QM9414	<i>Trichoderma reesei/Hypocrea jecorina</i>	SLU	Ascomycota	Cellulose, hemicellulose
E	ATCC 34541	<i>Phanerochaete chrysosporium</i>	SLU	<i>Basidiomycota white-rot</i>	Cellulose, hemicellulose, lignin
F	CTB 863A	<i>Trametes versicolor</i>	SLU	<i>Basidiomycota white-rot</i>	Cellulose, hemicellulose, lignin
G	90031-5P	<i>Ceriporiopsis subvermispora</i>	SLU	<i>Basidiomycota white-rot</i>	Cellulose, hemicellulose, lignin
H	M617	<i>Gloeophyllum tra-beum</i>	SLU	<i>Basidiomycota brown-rot</i>	Cellulose, hemicellulose, modifies lignin
I	CBS 457.96	<i>Trichoderma koningii</i>	Commercial	Ascomycota	Cellulose, hemicellulose
J	M71	<i>Phanerochaete chrysosporium</i>	SLU	<i>Basidiomycota white-rot</i>	Cellulose, hemicellulose, lignin
K	M89	<i>Trametes versicolor</i>	SLU	<i>Basidiomycota white-rot</i>	Cellulose, hemicellulose, lignin
L	M169	<i>Piptoporus betulinus</i>	SLU	<i>Basidiomycota brown-rot</i>	Cellulose, hemicellulose and modifies lignin



Table A.2. Total (TS) and volatile solids (VS) of all samples used in the BMP measurements

Sample	TS	VS	VS/TS	pH
	%	%	%	
<b>Straw/Pretreatment/Pretreatment time</b>				
Straw/ <i>P.ostreatus</i> /15d	36	34	94	
Straw/ <i>P.ostreatus</i> /20d	37	35	95	
Straw/ <i>P.ostreatus</i> /30d	38	36	95	
Straw/ <i>P.ostreatus</i> /35d	37	34	92	
Straw/ <i>P.eryngii</i> /35d	32	30	94	
Straw/ <i>P.ostreatus</i> -88d	54	51	94	
Straw/ <i>P.eryngii</i> /78d	38	36	95	
<b>Mushroom compost/sampling occasion/fungus</b>				
Mushroom compost/before/ <i>P.eryngii</i>	35	32	91	
Mushroom compost/after/ <i>P.eryngii</i>	50	43	86	
SV1/before/ <i>A. bisporus</i>	28	18	64	8.4
SV1/after/ <i>A. bisporus</i>	37	23	62	6.9
SV2/before/ <i>A. bisporus</i>	24	16	67	8.0
SV2/after/ <i>A. bisporus</i>	30	20	67	8.1
SV3/before/ <i>A. bisporus</i>	29	19	66	8.2
SV3/after/ <i>A. bisporus</i>	31	20	65	7.9
<b>Untreated straw</b>	94	91	97	
<b>Inocula</b>				
Inoculum A	3.4	2.3	68	
	2.9	2.0	69	
	3.9	2.7	69	
Inoculum B	3.6	2.2	61	

