Lignin Concentration During Incubation of Chicken Manure with Sawdust and Wheat Straw or Miscanthus Overgrown with *Pleurotos Ostreatus* Fungi

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Abstract In this study pre-treatment of wheat straw and Miscanthus with Pleurotus ostreatus fungi was performed. Mixtures of chicken manure with sawdust and fungi overgrown wheat straw or Miscanthus in different ratios (80:20 and 50:50) were incubated for 30 d at room temperature. The concentration of acid insoluble lignin was determined in different time intervals. In the samples with Miscanthus, the concentrations are 5-10 % lower than in the samples with wheat straw. In general, it is not clear that the lignin is degrading during the 30 d incubation. Furthermore, the concentrations of glucose and xylose were determined in initial samples and in samples after 30 d of incubation. On average, the concentration of glucose and xylose slightly decreased after 30 d of incubation. Reduction in the sugar concentrations is attributed to its consumption for the growth and development of fungi during the overgrowing of both substrates.

Keywords: chicken manure, *pleurotus ostreatus*, lignin, glucose, xylose.

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1 Introduction

In order to ensure the economic acceptability of the production of "clean" energy from renewable lignocellulosic materials, it is imperative to choose an affordable method or a combination of pre-treatment methods for disruption of the naturally recalcitrant carbohydrate-lignin shields (Paul & Dutta, 2018 and Wyman *et al.*, 2018). One of the possible methods is pre-treatment with fungi (Rouches *et al.*, 2016a). The pre-treatment efficiency depends on the strain of fungi selected and the origin of the biomass (Kucharska *et al.*, 2018). White-root fungi secrete enzymes (laccase and peroxidases) that partially break the bonds in polymer chains of lignocellulosic materials and thus make lignin more readily degradable for microorganisms (Rouches et al., 2016b). Known representatives of white-root fungi strains are Pleurotus ostreatus, Trametes versicolor, Heterobasidion annosum, Irpex lacteus, Phanerochaete chrysosporium, Coriolus versicolor, and others.

During our research we investigated the possibility of increased degradation of sawdust and lignocellulosic biomass in chicken manure using fungal pre-treatment, with the aim of increased biogas production. In this particular study, we tested *Pleurotus ostreatus* as a possible white-rot fungi for lignin biodegradation of two different co-substrates, wheat straw and Miscanthus.

2 Materials and methods

2.1 Materials

All the reagents and solvents used were of analytical grade. Dry pyridine (PYR), methanol (MeOH) and toluene were purchased from Merck (Germany), N-O-bis-trimethylsilyl trifluoroacetamide with 1 % of trimethylchlorosilane (BSTFA + 1 % TMCS) and $CaCO_3$ were from Fluka Chemie (Switzerland), D-glucose (99.5 %), D-xylose (98 %), and phenyl-β-D-glucopyranoside (99 %, ISTD) were supplied by Sigma-Aldrich (Germany). Wheat straw and Miscanthus were harvested in local fields in Slovenia. The chicken manure with sawdust used in this study was freshly collected from a biogas plant (Perutnina Ptuj, Draženci, Slovenia).

2.2 Methods

2.2.1 Fungi pre-treatment

Pre-treatment with white-rot wood decay fungi *Pleurotus ostreatus* was performed in 1 L glass jars filled with wheat straw or Miscanthus previously ground into 2 to 3 cm pieces and some water. The jars were sealed and autoclaved. After sterilization, the cooled substrate was inoculated under sterile conditions with cultured mycelium of an oyster mushroom (*Pleurotus ostreatus*, isolate *P.o.*/strain H35) overgrown on Potato Dextrose Agar (PDA) medium. After 3 weeks, the chicken manure with sawdust was mixed with pre-treated and ordinary wheat and pre-treated straw and ordinary Miscanthus at different mass ratios (80:20 and 50:50). The mixtures were further incubated for different periods of time.

2.2.1 Analysis

Acid-insoluble lignin was determined according to the Klasson method. Briefly, the extraction of a known amount of dry sample was performed in a Soxhlet apparatus using acetone as a solvent. The tube with the sample was transferred to the beaker. Water was added and the contents of the beaker were covered with foil and boiled for 1 h. Afterwards, the tube with the sample was removed from the beaker and dried. A known amount of extracted sample was put in a small beaker and hydrolyzed for 1 day using 72 % H_2SO_4. The solution was then transferred to larger beaker and diluted to a 3 % solution. The beaker was covered with the foil and allowed to boil for 4 h. The solution was cooled to room temperature and then filtered using suction filtration. The mass of dry sample left on the filter paper represents the amount of acid-insoluble lignin. The filtrate was taken and stored for later analysis of monosaccharides. TMS derivatives of monosaccharides were analyzed with a GC-FID system (GC HP Agilent 6890), equipped with a split/splitless injector (HP 6890 Autosampler Injector). Separation of the compounds was carried out on a fused silica capillary column (Agilent HP-5MS UI, 30 m × 0.32 mm i.d., 0.25 μm film thickness). 1 μL of the sample was injected in split mode (split ratio 7:1). Nitrogen 5.0 (Messer d.o.o.) at a flow rate of 0.2 mL min^{-1} was used as the carrier gas. The injector temperature was 250 °C. The temperature program of the column was as follows: 1 min held at 70 °C, then raised to 200 °C

using 2 °C min^{-1} rate; afterwards, the temperature was increased to 320 °C at 10 °C min^{-1} rate and, finally, held there for 3 min. The total analysis time was 81 min. The flame ionization detector was operated at 250 °C. The gas flows were as follows: hydrogen 30 mL min^{-1}, synthetic air 300 mL min^{-1}, nitrogen 10 mL min^{-1}. All gases used had a purity of 5.0 (Messer d.o.o.). (Medeiros & Simoneit, 2007, Mejanelle *et al.*, 2002, Ruiz-Matute *et al.*, 2011)

3 Results

3.1 Concentration of acid-insoluble lignin

The incubation of mixtures of chicken manure with sawdust and pre-treated and ordinary wheat straw or pre-treated and ordinary Miscanthus lasted for different periods of time (abbreviations: CMS:S - Chicken Manure with Sawdust to ordinary wheat Straw, CMS:*P.o.* - Chicken Manure with Sawdust to pre-treated wheat straw with *Pleurotus ostreatus* fungi and CMS:M - Chicken Manure with Sawdust to ordinary Miscanthus, CMS:*P.o.* - Chicken Manure with Sawdust to pre-treated Miscanthus with *Pleurotus ostreatus* fungi). The samples were taken t = (0, 5, 9, 14, 19, 23 and 30) day. The concentrations of acid-insoluble lignin for all different mixtures and incubation periods are summarized in Table 1.

It can be seen from Table 1 that there is no clear dependence between lignin concentration and incubation time, regardless of whether the substrate (wheat straw or Miscanthus) was pre-treated or not. The lowest lignin concentration, w_L = 18.10 %, was obtained for the wheat straw for two mixtures, CMS:S = 80:20 after 5 d and CMS:*P.o.* = 50:50 after 30 d, and the highest value, w_L = 25.90 %, was obtained for the mixture of CMS:S = 50:50 after 23 d. The concentrations of acid-insoluble lignin for the Miscanthus samples were 5-10 % lower than those for the wheat straw samples. The lowest value of lignin concentration (w_L = 9.20 %) was obtained for Miscanthus for the mixture of CMS:*P.o.* = 80:20 after 9 d and the highest value (w_L = 15.60 %) for the mixture of CMS:*P.o.* = 50:50 after 30 d.

Additionally, the concentrations of monosaccharides were determined in the filtrate that was taken during the determination of acid-insoluble lignin according to the Klasson method. The concentrations of glucose and xylose were determined in the samples after 0 and 30 d of incubation.

Table 3 summarizes concentrations of glucose and xylose for all different mixtures of chicken manure with sawdust and pre-treated and ordinary Miscanthus. We can see that after 30 d of incubation, the concentrations of both monosaccharides, regardless of the substrate used, are lower than at the beginning. We could attribute that to the consumption of glucose and xylose during the incubation period. Fungi could use them for the purposes of nutrition, growth and development. Nevertheless, the concentrations are also lower for samples of substrates not pre-treated with fungi. The plausible explanation is that the microorganisms located in chicken manure consume sugar similar to the fungi.

Table 1: Concentrations of acid-insoluble lignin.

	Wheat straw			Miscanthus		
t / d	SUBSTRATE	MASS RATIO	w_L / %	SUBSTRATE	MASS RATIO	w_L / %
0	CMS:*P.o.*	80:20	20.10	CMS:*P.o.*	80:20	11.80
		50:50	20.70		50:50	15.00
	CMS:S	80:20	20.90	CMS:M	80:20	11.20
		50:50	23.40		50:50	14.00
5	CMS:*P.o.*	80:20	19.90	CMS:*P.o.*	80:20	13.50
		50:50	20.80		50:50	14.00
	CMS:S	80:20	18.10	CMS:M	80:20	9.50
		50:50	19.40		50:50	15.10
9	CMS:*P.o.*	80:20	21.30	CMS:*P.o.*	80:20	9.20
		50:50	22.60		50:50	15.50
	CMS:S	80:20	24.20	CMS:M	80:20	10.30
		50:50	23.20		50:50	13.80
14	CMS:*P.o.*	80:20	25.50	CMS:*P.o.*	80:20	15.20
		50:50	20.80		50:50	13.30
	CMS:S	80:20	22.10	CMS:M	80:20	11.60
		50:50	21.70		50:50	15.30
19	CMS:*P.o.*	80:20	21.10	CMS:*P.o.*	80:20	13.20
		50:50	19.60		50:50	14.50
	CMS:S	80:20	26.30	CMS:M	80:20	13.10
		50:50	23.08		50:50	15.50
23	CMS:*P.o.*	80:20	24.80	CMS:*P.o.*	80:20	10.30
		50:50	20.10		50:50	12.50
	CMS:S	80:20	25.90	CMS:M	80:20	13.50
		50:50	25.90		50:50	15.00
30	CMS:*P.o.*	80:20	22.30	CMS:*P.o.*	80:20	11.40
		50:50	18.10		50:50	15.60
	CMS:S	80:20	22.10	CMS:M	80:20	11.90
		50:50	24.70		50:50	14.90

Table 2: Concentrations of glucose and xylose for wheat straw samples.

t / d	Wheat straw			
	SUBSTRATE	**MASS RATIO**	w_G / %	w_X / %
0	CMS:*P.o.*	80:20	18.24	10.13
		50:50	16.74	6.11
	CMS:S	80:20	14.72	7.96
		50:50	20.46	10.96
30	CMS:*P.o.*	80:20	14.14	6.56
		50:50	16.55	6.46
	CMS:S	80:20	12.73	3.87
		50:50	15.66	7.09

The concentration of glucose and xylose for all different mixtures of chicken manure with sawdust and pre-treated and ordinary wheat straw are summarized in Table 2.

Table 3: Concentrations of glucose and xylose for Miscanthus samples.

t / d	Miscanthus			
	SUBSTRATE	**MASS RATIO**	w_G / %	w_X / %
0	CMS:*P.o.*	80:20	14.32	3.94
		50:50	39.08	11.27
	CMS:M	80:20	24.08	6.98
		50:50	25.36	7.05
30	CMS:*P.o.*	80:20	10.01	3.29
		50:50	14.58	4.74
	CMS:M	80:20	7.56	1.89
		50:50	12.41	3.46

4 Conclusion

In analysing the experimental data set, it was found that the concentration of acid-insoluble lignin for Miscanthus samples was 5-10 % lower than that for the wheat straw samples. In general, it is not clear that lignin in either of these two co-substrates would degrade during the 30 d of incubation.

To confirm this claim, we determined the glucose and xylose concentrations by GC-FID in the filtrate after the Klasson lignin was found. Concentrations of both monosaccharides decreased slightly after 30 d of incubation. This trend could be attributed to the consumption of sugars for the growth and development of fungi during the incubation of both substrates.

The results of this study showed that pre-treatment of wheat straw or Miscanthus with selected *Pleurothus ostreatus* white-rot fungi did not cause the expected biodegradation of lignocellulosic material investigated in this particular study.

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