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Effect of enzymatic pretreatment of various lignocellulosic substrates on production of phenolic compounds and biomethane potential

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HIGHLIGHTS

- An enzymatic pretreatment results in a significant increased release of phenolics.
- A variable pattern in release of phenolic compounds was detected among substrates.
- A linear relation between a broad lignin range and BMP was established in one study.
- Miscanthus and willow having higher lignin concentration, had significant lower BMP.

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ABSTRACT

Pretreatment of lignocellulosic biomass is necessary to enhance the hydrolysis, which is the rate-limiting step in biogas production. Laccase and versatile peroxidase are enzymes known to degrade lignin. Therefore, the impact of enzymatic pretreatment was studied on a variety of biomass. A significant higher release in total phenolic compounds (TPC) was observed, never reaching the inhibiting values for anaerobic digestion. The initial concentration of TPC was higher in the substrates containing more lignin, miscanthus and willow.

The anaerobic digestion of these two substrates resulted in a significant lower biomethane production (68.8–141.7 Nl/kg VS). Other substrates, corn stover, flax, wheat straw and hemp reached higher biomethane potential values (BMP), between 241 and 288 Nl/kg VS. Ensilaged maize reached 449 Nl/kg VS, due to the ensilation process, which can be seen as a biological and acid pretreatment. A significant relation (R^2 = 0.89) was found between lignin content and BMP.

1. Introduction

Alternative green energy can form a solution for global problems such as climate change and the diminishing amount of available fossil fuels (Divya et al., 2015). Biogas production via anaerobic digestion of agricultural waste streams is a cheap alternative energy source. These agricultural waste streams can be lignocellulose-rich materials which contain the polymers cellulose, hemicellulose and lignin (Theuretzbacher et al., 2015). The ratio of these polymers differs between different types of substrate. Lignin is a major natural source of phenolic compounds, containing variously linked phenylpropane units, such as hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units (Jung et al., 2015). Phenolic compounds are desired to produce chemicals with aromatics and their derivatives after catalytic pyrolysis of lignin (Ma et al., 2012).

A higher lignin content increases the release of phenolic compounds during degradation and decreases the availability of cellulose. Examples of lignocellulosic biomass are corn stover, wheat straw, flax, hemp, miscanthus and willow. Table 1 presents the compositions of such lignocellulosic biomass according to recent literature. As lignin is the most recalcitrant polymer and as it forms a barrier around the cellulose and hemicellulose, lower BMP of these substrates are reported, as shown in Table 1. Although a relation between lignin content and BMP is expected, the determination of lignin concentration together with BMP values for this range of agricultural residues has not yet been described under the same experimental conditions, i.e. in one study. Moreover the large range of lignin concentration reported for the same substrate in different studies vary with the selected parts of the plant or time of harvest, thus complicating comparisons between studies, if both lignin and BMP are not characterized on the same sample.

In the biogas production process from such lignocellulosic biomass four steps occur: hydrolysis, acidogenesis, acetogenesis and

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Table 1
BMP and the composition of cellulose, hemicellulose and lignin of the selected substrates according to recent literature and as determined in this study (measured values).

Substrate	Measured values in this study		Literature values				References
	Lignin (g/100 g)	BMP (Nl/kg VS)	Cellulose (g/100 g)	Hemicellulose (g/100 g)	Lignin (g/100 g)	BMP (Nl/kg VS)*	
Corn stover	4.5	192–288	32.6–40.5	13.7–31.2	5.0–25.9	317–363	Schilling et al. (2012)#, Tuyen et al. (2013)#, Raposo et al. (2012)*
Wheat straw	6.0	200–251	35.1–39.2	25.6–26.1	7.5–15	227–333	Chandra et al. (2012)#, Krishania et al. (2012)#, Raposo et al. (2012)*
Flax	8.6	207–244	33.3	21.9	26.0	212	Ross and Mazza (2010)#, Koçar and Civas (2013)*
Hemp	9.2	184–248	62.6	17.2	9.8	355–409	Amaducci et al. (2000)#, Koçar and Civas (2013)*
Miscanthus	12.0	129–142	25.8–48.5	19.1–27.0	20.5–30.0	179–218	Xu et al. (2014)#, Saleh et al. (2013)#, Koçar and Civas (2013)*
Willow	17.0	69–97	37.3	17.9	25.3	130–370	Mante et al. (2014)#, Raposo et al. (2012)*

*Study determining the BMP value; #study determining C/H/L content.

methanogenesis. The hydrolysis is the rate limiting step in which the lignin barrier is broken down (Appels et al., 2008). Known methods for improving the hydrolysis step are acidic, mechanical, thermal and biological pretreatments (Pilli et al., 2014; Li et al., 2015). Acidic, mechanical and thermal pretreatments have been shown effective on lignin degradation, however these techniques are more costly and result in a higher production of inhibitory compounds such as *p*-coumaric acid and 4-hydroxybenzoic acid (Wang et al., 2013; Kratky and Jirout, 2015). Biological pretreatments using white rot fungi to breakdown lignin by producing enzymes such as laccase or versatile peroxidase can be a cheap alternative to improve the hydrolysis step, and prohibit the formation of high concentrations of inhibiting compounds (Dong et al., 2013). Some studies even have shown a decrease of phenolic compounds due to the use of laccase (Jönsson et al., 1998; Ramirez et al., 2014; Schroyen et al., 2014).

In this paper the effect of an enzymatic pretreatment, being a combination of laccase and versatile peroxidase, on different plant biomass with different lignin concentrations for BMP values is investigated. Doing so, a relation between lignin content and BMP is determined. Moreover, the release of inhibitors like total phenolic compounds as well as several individual phenolic compounds is determined.

2. Methods

2.1. Substrates

Substrates were chosen based on their lignin content as reported in literature (Table 1). Hemp, flax and corn stover were obtained from InAgro vzw (Roeselare, Belgium). Miscanthus and willow were acquired from the Institute for Agricultural and Fisheries Research (Merelbeke, Belgium), while ensilaged maize and wheat straw were collected from a local farm (Den Hoef, Maaseik, Belgium). The lignin values were determined according to Van Soest et al. (1991).

2.2. Chemicals

o-Coumaric acid, *p*-coumaric acid, ferulic acid, gallic acid, vanillin, syringic acid, sinapic acid, vanillic acid, hydroxymethylfurfural (HMF), dinitrosalicylic acid, sodium chloride (NaCl), sodium malonate ($C_3H_2O_4Na_2$), hydrogen peroxide (H_2O_2), 4-hydroxybenzoic acid ($C_7H_6O_3$), potassium dichromate ($K_2Cr_2O_7$), potassium hydrogen phthalate ($C_8H_5KO_4$) and laccase enzyme were obtained from Sigma–Aldrich (Bornem, Belgium) while versatile peroxidase was attained from Jena Bioscience (Jena, Germany). Silver sulfate (Ag_2SO_4), hydroxylamine hydrochloride (H_4ClNO), sulfuric acid (H_2SO_4), furfural and Folin–Ciocalteu's phenol reagent were acquired from ChemLab (Zedelgem, Belgium). Tween 80 was purchased from Acros Organics (New Jersey, USA), sodium carbonate (Na_2CO_3) and mercury(II) sulfate ($HgSO_4$) were obtained from Merck (Darmstadt, Germany) and 1-hydroxybenzotriazole was purchased from Janssen Pharmaceuticals (Beerse, Belgium). HPLC-grade methanol (MeOH), HPLC-graded water, acetic acid, glucose, potassium sodium tartrate ($KNaC_4H_4O_6{\cdot}4H_2O$) were purchased from VWR (Leuven, Belgium). All chemicals were used as provided.

2.3. Enzymatic pretreatment

The substrates were cut in fragments of ±0.5 cm to achieve the average size of ensilaged maize. An acetate buffer (0.1 M, pH = 4.5) was used to submerge 9 g of the substrate, the enzymes and the accompanying additives in a total of 180 ml. The enzymes used were laccase derived from *Trametes versicolor* (2 U/g substrate; 1 U is defined as the release of 1 μmol catechol/min at pH 6 and 25 °C), and versatile peroxidase from *Bjerkandera adusta* (1.5 U/g substrate; 1 U is defined as the release of 1 μmol Mn(II)/min at pH 4.5 and 25 °C). Additives were added to increase enzyme activity. Per gram biomass, 4 mg 1-hydroxbenzotriazole, 58 mg sodium-tartrate, 148 mg sodium-malonate, 156 μl H_2O_2 30% (w/w) in H_2O and 111 μl Tween 80 was added (Frigon et al., 2012; Schroyen et al., 2014). A control treatment, without the enzymes and additives, was included. An incubation at 30 °C for 0, 6 and 24 h was performed for the control flasks, while an incubation at 30 °C for 6 and 24 h was setup for the flasks containing the enzymatic pretreatment. During the incubation the flasks were continuously shaken at 60 rpm. After the incubation periods, the substrates were filtered at room temperature using a filter paper (VWR, Leuven, Belgium) with pore sizes of 5–13 μm. The solid residue (further denoted as solid fraction) was kept to analyze total suspended solids (TSS) and volatile suspended solids (VSS), to extract phenolic compounds and to determine the biomethane potential (BMP) as described in Schroyen et al. (2014). The filtrate (further denoted as liquid fraction) was used to analyze the phenolic compounds, biologic oxygen demand (BOD) and chemical oxygen demand (COD) (Schroyen et al., 2014). TSS, VSS, BMP, BOD and COD analysis were performed according to standard methods (APHA, 2005). For each treatment, three independent replicates were performed. To measure an increase in parameters during time of incubation, the results from the control flasks at the start of the incubation are subtracted from the results of the other samples. Extra control flasks with only enzymes and additives, or only buffer solution were made to correct for their background from the enzymatic pretreated samples in order to compensate the interferences of the additives.