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Strategies for Enhanced Biogas Generation through Anaerobic Digestion of Forest Material – An Overview

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Incorporation of biofuels into the existing selection of fuels is a very important measure to slow down environmental destruction and to counter the imminent fossil fuel shortage. Biogas is a very interesting option for use in both electricity and heat production, and also as a fuel for vehicles. The positive fuel characteristics of biogas and the high yields of biogas obtained from traditional raw materials (*e.g.*, animal manure) have resulted in operation of several commercial units around the globe. On the other hand, there is an increased demand for biogas production which, for the need to be met, should have renewable resources incorporated into it. Forest materials are an interesting candidate, and there is a rising interest in the research and industrial communities to exploit them as raw materials for anaerobic digestion in biogas production. In this review article, we aim to give the reader an insight into the most recent processes for conversion of various sources of forest materials into biogas.

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INTRODUCTION

The rapid increase in industrialization that was observed during the last century, together with the population increase, has resulted in an increase in the need for energy (Matsakas *et al.* 2014a; Sarris *et al.* 2014). Currently, the main source of energy is fossil resources (such as petroleum) (Matsakas *et al.* 2014b). The use of fossil fuels as an energy source is not only unsustainable, but it also leads to environmental problems such as the release of greenhouse gases (GHGs) and other potentially harmful compounds (Bonturi *et al.* 2015). Moreover, the use of fossil resources has resulted in dependence of the vast majority of countries on the importation of fuels, which can result in energy insecurity. To face these issues, novel renewable sources of energy must be used. These forms of biofuels should preferably be produced with technology based on local raw materials, which results in energy security and the creation of new jobs, *i.e.*, enabling the transfer to a biobased economy. The most common biofuels that are currently used are the liquid fuels ethanol and biodiesel, and the gaseous fuel methane. During their production, it is very important that the raw materials that are used are not also food sources or compete with land used for food production. The use of edible resources for the production of biofuels has already been severely criticized (Nitsos *et al.* 2015), as it has led to an increase in their prices in the past.

The main uses of biogas are as vehicle fuel and for electricity production (Jeihanipour *et al.* 2013). To produce electricity, biogas is burned in special equipment called CHP (combined heat and power). On the other hand, for use as a fuel for vehicles,

biogas must be upgraded by removing the carbon dioxide (CO_2) and other gases (*e.g.*, hydrogen sulfide (H_2S)) and subsequently increasing the concentration of methane (CH_4), which results in an increase in the energy content of the biogas. Different techniques are employed to increase the methane content, such as chemical adsorption, activated carbon, scrubbing, and biological oxidation (Abatzoglou and Boivin, 2008). Biogas production, according to the European Biomass Industry Association (www.eubia.org), in EU25 during 2012 reached 12,090 ktoe (thousand tonnes of oil equivalent), with Germany having the leading role in the production (6,416 ktoe) and with the UK (1,811 ktoe) and Italy (1,178 ktoe) following. According to Statistics Sweden (scb.se), Sweden has already established a biomethane-based car market with a total of 40,095 gas/gas flexifuel vehicles in 2014; and among them 8,150 are methane gas vehicles.

Biogas, as will be discussed later, is produced through anaerobic digestion of organic materials. The process of anaerobic digestion has a positive environmental impact, as it prevents the disposal of organic wastes in landfills. Disposal of organic material in landfills can result in uncontrolled release of gases into the environment. These gases include methane, which is considered to present 20 to 23 times higher greenhouse gas potential compared to carbon dioxide (Browne and Murphy 2013; Zheng *et al.* 2014). Moreover, the landfills also generate leachates, which result in contamination of underground waters. Finally, another positive characteristic of anaerobic digestion is the generation of digestate. Digestate is the remaining solid fraction after the digestion, and it is rich in nitrogen, potassium, and phosphorus, and has peculiar rheological characteristics; these properties make it a very efficient biofertilizer (Liu *et al.* 2009; Adu-Gyamfi *et al.* 2012; Kafle *et al.* 2014).

Biogas as Fuel

One of the attractive advantages of using biogas as a vehicle fuel is that it does not require extensive modification of the vehicle. The technology for the conversion of a gasoline fueled vehicle to operate with methane is already proven, and the modification of a spark ignition engine is easy and can be done by the addition of a second fueling system (Aslam *et al.* 2006). The cost of this conversion varies depending on the size and type of the vehicle, the type of engine, the size of the methane tank, the labor cost, and the country in which the modification will take place. The use of biogas as a fuel contributes to a reduction in GHGs emissions and air pollution, as it has negligible emission of dust and particles, practically no GHGs release, and lower emission of sulfur compounds, carbon monoxide, nitrogen oxides, and hydrocarbons (Swedish Gas Association 2011). Despite the very positive characteristics of biogas as a fuel and the fact that it is more important to use the produced biogas for vehicle fuel rather than electricity production (because there is a wide availability of "green" alternatives, such as wind and solar power), only relatively small amounts of biogas are directed to the transportation sector (Naik *et al.* 2010). In terms of resource efficiency, biogas is generally considered to have a better ratio of energy output to input than ethanol (Jeihanipour *et al.* 2013), reaching a ratio of up to 28 (Zheng *et al.* 2014).

Production and use of any form of renewable energy to be used as vehicle fuel, always has to compete with the prices of fossil fuels (*e.g.* petroleum). After approximately the middle of 2014, the prices of fossil fuels have been significantly decreased compared to the previous years and this has increased the pressure of renewable alternatives. Although the economic value of a fuel is an important factor, other issues have to be taken under serious consideration. Use of renewable fuels is very important from an

environmental point of view as it results in the decrease of the release of GHGs and other air pollutants. Moreover, the use of renewable sources for the production of energy results in an infinite supply, whereas use of fossil fuels is going to face a shortage in the future. Another benefit of the production of renewable fuels is the ability to use 'local' raw materials (like forest materials in Sweden), which will boost the local economy and also decrease the dependence of a country in the import of fuels.

The Biogas Production Process

Methane is a gaseous energy carrier that is produced from the anaerobic digestion of organic materials. Anaerobic digestion is a complex process that involves the degradation of different organic molecules (*e.g.*, carbohydrates, proteins, and fats) to methane in different steps (Matsakas *et al.* 2014c). These steps involve the initial degradation of complex molecules (*e.g.*, polysaccharides) to simple ones (*e.g.*, monomeric sugars) followed by conversion of these simple molecules to a variety of volatile fatty acids (VFAs) during the acidogenesis step (Fig. 1). In the next step (acetogenesis), the VFAs that are longer than acetic acid are converted to acetic acid, CO_2, and H_2, and during the last step of the digestion the VFAs are converted to CH_4 and CO_2 by methanogens (Adu-Gyamfi *et al.* 2012). The first stage of VFAs production is a faster process than the methanogenesis. If the whole process becomes imbalanced, accumulation of VFAs may occur, which results in the acidification and subsequent inhibition of methanogenesis (Griffin *et al.* 1998).
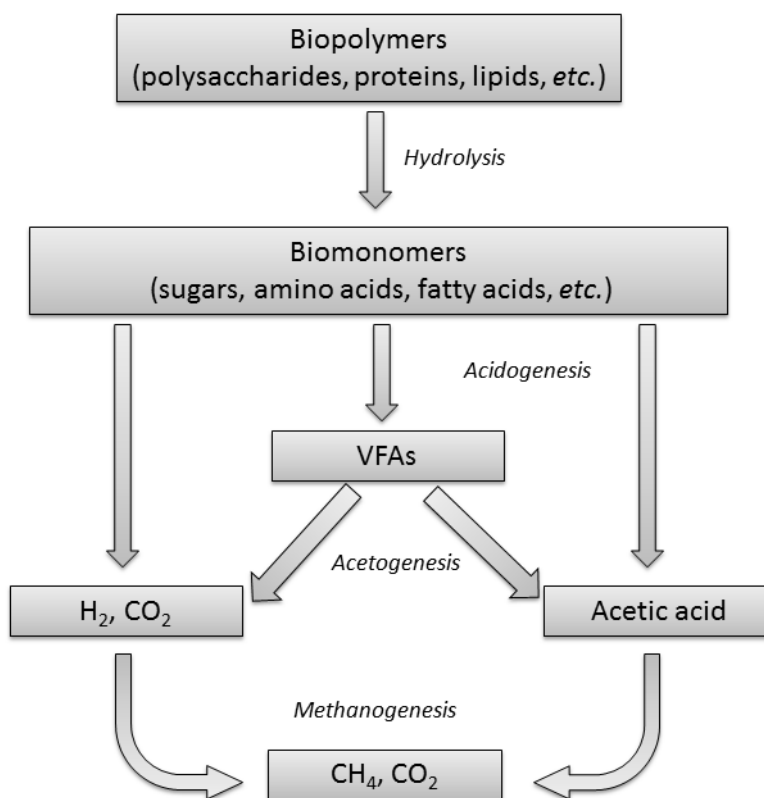

Fig. 1. Steps involved in the biological degradation of organic materials to biogas

Biogas production normally takes place under psychrophilic (< 25 °C), mesophilic conditions (25 to 35 °C), or thermophilic conditions (45 to 60 °C) (Liu *et al.* 2009; Zhou *et al.* 2012). Generally, the thermophilic process is considered to be more beneficial, as it leads to higher methanogenic activity and therefore a faster process, and it also prevents microbial contamination (Lesteur *et al.* 2010; Xia *et al.* 2013). The main materials that are used for anaerobic digestion are animal manure, food waste, and slaughterhouse waste. Anaerobic digestion of these materials is very efficient, and high yields have already been achieved (Hill and Bolte 2000; Nielsen *et al.* 2004; Banks *et al.* 2011; Palatsi *et al.* 2011; Nagao *et al.* 2012). The increasing demand for biogas has resulted in the need for incorporation of new resources for anaerobic digestion. Lignocellulosic biomass could serve as a novel renewable material during anaerobic digestion.

LIGNOCELLULOSIC BIOMASS

Lignocellulosic biomass is an abundant renewable raw material that can be used for the production of biofuels (Xia *et al.* 2013). It has been estimated that the annual production of biomass can reach 150 billion tons of organic matter (100 billion tons of these concern land biomass and 50 billion tons aquatic biomass) (Naik *et al.* 2010). The productivity of biomass formation (estimated in dry ash free biomass) is between 7.5 and 15 tons/ha/year (Datta *et al.* 2011). Sources of low-cost lignocellulose can be forest residues, agricultural residues, and even solid waste from the industrial and domestic sectors (McKendry 2002; Tomás-Pejó *et al.* 2008). Apart from this, biomass is considered to play a very important role in the reduction of atmospheric CO_2 as it both acts as a reservoir of CO_2 and also substitutes the use of fossil fuels (Ciubota-Rosie *et al.* 2008). It is calculated that a replacement of just 5% of the liquid fossil fuels by biomass-based liquid fuels will have the potential of decreasing the CO_2 emissions by 0.4 Gt/year (Sridhar and Hill 2011).

Lignocellulose is a generic term used for describing the main constituents found in plant cell walls. It mainly consists of cellulose, hemicellulose, lignin, extractives, and inorganic materials (Taherzadeh and Karimi 2008). The composition of the main components (cellulose, hemicellulose, and lignin) varies depending on the source of the lignocellulose, with an average composition of cellulose being 35% to 50%, hemicellulose 20% to 35%, and lignin 5% to 30% (Lynd *et al.* 2002). Cellulose is a linear crystalline homopolymer of β-1,4-D-glucose with a high degree of polymerization (Taherzadeh and Karimi 2008; Zheng *et al.* 2014). Because of the nature of the bonds formed between different chains, cellulose has a very stable structure that is difficult to decompose. Different parts of cellulose have varying degrees of polymerization and are classified as being crystalline or amorphous (Sun *et al.* 2008). Hemicellulose, on the other hand, is a heteropolymer consisting of both pentoses (xylose and arabinose) and hexoses (glucose, mannose, rhamnose, and galactose) together with uronic acids (glucuronic acid, galacturonic acid, and methyl glucuronic acid) (Zheng *et al.* 2014). Hemicellulose is easier to hydrolyze than cellulose, and it has a lower degree of polymerization (Saha 2003). The role of hemicellulose is to protect cellulose from enzymatic degradation. Finally, lignin is a complex heteropolymer consisting of phenylpropane units (such as sinapyl alcohol, *p*-coumaryl alcohol, and coniferyl alcohol) (Nigam and Singh 2011; Stamatelou *et al.* 2012). Lignin is responsible for giving integrity and structural rigidity, and for prevention of swelling of lignocelluloses (Taherzadeh and Karimi 2008; Zheng *et al.* 2014).

High amounts of residual wood biomass are produced annually by the wood industry (the pulp and paper industry, sawmills, *etc.*) (Garcìa-Pérez *et al.* 2007), which could serve as raw material for anaerobic digestion of plants. Woods are classified as softwoods and hardwoods, depending on their structure. Softwoods (with spruce and pine being characteristic representatives) have an approximate composition of 43% to 45% cellulose, 20% to 23% hemicellulose, and 28% lignin, with the hemicellulose primarily consisting of mannose (Galbe and Zacchi 2002). On the other hand, the hemicellulose present in hardwoods (*e.g.*, birch and willow) is approximately 25% of the dry mass and primarily consists of xylose (Lawford and Rousseau 1991). Softwoods and hardwoods also show differences in the structure of lignin. More specifically, the lignin of softwoods consists mostly of guaiacyl units, whereas the lignin of hardwoods is mostly a mixture of guaiacyl and syringyl units (Taherzadeh and Karimi 2008).

The Need for Pretreatment

The complex structure of lignocellulose makes it a very stable compound that is very difficult to hydrolyze. Generally, the degree of hydrolysis of cellulose is affected by different factors, including the crystallinity, the degree of polymerization, the levels of hemicellulose and lignin present, the degree of acetylation of hemicellulose, and the amount of accessible surface area (Kim and Holtzapple 2006; Parawira 2012). These factors have a negative effect on the degree of decomposition of cellulose and hemicellulose, resulting in very low levels of product when untreated lignocellulosic materials are used. To overcome these barriers, some sort of pretreatment process must be used prior to any microbial conversion (Demirbas 2008; Matsakas and Christakopoulos 2015). For a pretreatment to be considered efficient, it should result in cellulose that is more easily hydrolysable, low levels of degradation of sugars and formation of inhibitor compounds—and the process should also have low operational costs and low environmental impact. Although generally pretreatment contributes to the cost of the process, it is absolutely necessary in order to achieve high methane yields and it was shown recently by Kabir *et al.* (2015) that biogas production from organosolv pretreated forest biomass can be financially viable.

There is a wide variety of pretreatment processes available in the literature, which are classified as physical, physicochemical, chemical, and biological (Mosier *et al.* 2005; Matsakas and Christakopoulos 2013; Xiros *et al.* 2013). The efficiency of a pretreatment strongly depends on the source of lignocellulosic biomass used, and for this reason there is no universal solution that can be applied to every biomass.

SOFTWOODS

Spruce

Spruce (*e.g.*, *Picea abies*) is an important example of softwood. Untreated spruce is not very efficient for use in anaerobic digestion, as the methane yield obtained was as low as 30 mL CH_4/g VS (volatile solids) (Mirahmadi *et al.* 2010; Mohsenzadeh *et al.* 2012), whereas the highest yield obtained was only 85 mL CH_4/g VS (Gao *et al.* 2013).

Hydrothermal pretreatment with sulfur dioxide (SO_2) was found by us to be very efficient for treating spruce; a methane yield of 276 mL CH_4/g VS (Table 1) was reached when spruce was treated with SO_2 at 212 °C for 4 to 8 min, with a pH of 1.6 to 1.8 at the end of the pretreatment (Matsakas *et al.* 2015).

Table 1. Different Sources of Softwood Materials and Yield of Biogas Obtained during Anaerobic Digestion

Substrate	Pretreatment	Enzymes	Type of digestion	Methane yield (mL CH_4/g VS)	Reference
Spruce	Hydrothermal with SO_2	10 IU/g laccase and 30 FPU/g cellulolytic	Thermophilic	276	Matsakas *et al.* 2015
Spruce	Steam refining with SO_2	-	Mesophilic	304[*]	Janzon *et al.* 2014
Spruce	Alkaline	-	Thermophilic	50	Mirahmadi *et al.* 2010
Spruce	Alkaline/thiourea	-	Thermophilic	210	Mohsenzadeh *et al.* 2012
Spruce	Ionic liquids	-	Thermophilic	245	Teghammar *et al.* 2012
Spruce	Ionic liquids	-	Mesophilic	141	Gao *et al.* 2013
Pine	-	-	Mesophilic	54	Brown *et al.* 2012
Pine	Hydrothermal with SO_2	10 IU/g laccase and 15 FPU/g cellulolytic	Thermophilic	180	Matsakas *et al.* 2015
Pine needle leaves	Untreated	-	Mesophilic	213	Salehian and Karimi 2012
Pine branches	Alkaline	-	Mesophilic	98	Salehian and Karimi 2012
Pine cones	Alkaline	-	Mesophilic	75	Salehian and Karimi 2012
Pine bark	Alkaline	-	Mesophilic	107	Salehian and Karimi 2012
Pine	Alkaline	-	Mesophilic	178	Salehian *et al.* 2013
Pine chips	Ionic liquids	-	Mesophilic	143	Shafiei *et al.* 2014
Pine powder	Ionic liquids	-	Mesophilic	224	Shafiei *et al.* 2014
Pine	Organosolv with sulfuric acid	-	Mesophilic	54	Mirmohamadsadeghi *et al.* 2014
Japanese cedar	Refiner	-	Mesophilic	26[**]	Take *et al.* 2006
Japanese cedar	Steaming and refiner	-	Mesophilic	45[**]	Take *et al.* 2006
Japanese cedar	Biological	-	Mesophilic	43[**]	Take *et al.* 2006
Japanese cedar	Steam explosion	-	Mesophilic	180[**]	Take *et al.* 2006
Mixture of spruce and pine	Ionic liquids	-	Thermophilic	170	Kabir *et al.* 2013
Mixture of spruce and pine	Ionic liquids	-	Thermophilic	150	Kabir *et al.* 2014
Mixture of spruce and pine	Ionic liquids	-	Mesophilic	109	Aslanzadeh *et al.* 2014
Mixture of spruce and pine	Organosolv	-	Thermophilic	340	Kabir *et al.* 2015

[*]Given as total biogas yield and not methane yield.
[**]Unclear whether this was total methane volume or methane yield.

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To achieve this high yield, enzymatic detoxification with laccase (15 U/g for 12 h prior to digestion) and addition of cellulolytic enzymes (30 FPU/g) simultaneously with the digestion was necessary. On the other hand, when spruce was pretreated under milder conditions (200 °C for 4 to 8 min and pH after pretreatment between 1.8 and 2.0) the methane yield was slightly lower, reaching 259 mL CH_4/g VS. To achieve this yield, addition of 30 FPU/g of cellulolytic enzymes was necessary, whereas there was no need for detoxification. In another study, when steam refining pretreatment was used at 220 °C for 5 min with the addition of 2.4% SO_2, the biogas yield that was obtained reached 304 mL/g VS (Janzon *et al.* 2014). This is difficult to compare with other studies, where the results are given in mL CH_4/g VS, as the methane content of biogas can vary. According to these authors, the methane content of biogas varies between 50% and 80%, which implies a methane yield between 152 mL CH_4/g VS and 243 mL CH_4/g VS approximately.

Apart from the thermal pretreatment with the use of inorganic acids, other researchers have evaluated the use of alkali during the pretreatment process. For example, Mirahmadi *et al.* (2010) pretreated spruce with 7% NaOH for 2 h at 5 °C. These pretreatment conditions were not as efficient as with acid, as the methane yield of the alkali-pretreated spruce reached only 50 mL CH_4/g VS. In another study, the same research group supplemented the NaOH with 12% thiourea and treated spruce at −15 °C for 16 h. Addition of thiourea had a positive effect on the methane yield, which reached 210 mL CH_4/g VS (Mohsenzadeh *et al.* 2012).

Finally, in some other studies, another strategy was evaluated, whereby the pretreatment involved the use of ionic liquids. For example, Teghammar *et al.* (2012) evaluated the effect of milling of spruce chips on the methane yield, both untreated and pretreated with the ionic liquid N-methylmorpholine-N-oxide (NMMO) at 130 °C for 15 h. They found that milling of the chips was necessary for both treated and untreated materials, whereas the use of ionic liquid pretreatment improved the methane production from the chips from 11 to 125 mL CH_4/g VS and that from the milled chips from 66 to 245 mL CH_4/g VS. On the other hand, a lower methane yield was obtained by Gao *et al.* (2013) using [C_4mim]Cl/DMSO (1-*N*-butyl-3-methlyimidazolium chloride/dimethyl sulfoxide) to treat spruce at 120 °C for 2 h. The highest methane yield that they found was 141 mL/g VS, which was almost half the yield reported with the use of NMMO. However, it is not always easy to compare the yields between different studies, as several factors can affect the methane yield, such as the source of inoculum and the protocol used for methane yield determination.

Pine

Pine is also an important representative of softwoods, and it can be found in most of the countries in the northern hemisphere. When pine is used untreated for anaerobic digestion, the methane yield obtained is normally low. Brown *et al.* (2012) evaluated untreated pine as raw material for both liquid and solid anaerobic digestion. Liquid digestion was more efficient, but the methane yield was only 54 mL CH_4/g VS (Table 1). The corresponding yield for solid-state digestion was 17 mL CH_4/g VS.

Hydrothermal pretreatment (210 to 215 °C for 5 min) with SO_2 as catalyst was found to be efficient for the pretreatment of pine, resulting in 180 mL CH_4/g VS (Matsakas *et al.* 2015). To achieve this yield, detoxification with laccase (10 U/g) and addition of cellulolytic enzymes (15 FPU/g) was necessary.

Alkaline pretreatment was employed by Salehian and Karimi (2012) to pretreat different parts of pine trees (needles (leaves), branches, cones, and bark). Pretreatment was

done with 8% NaOH for either 60 min at 0 °C or 10 min at 100 °C. The use of alkaline pretreatment improved the methane yield in all the tree parts, except for needles—where the methane yield actually decreased compared to the untreated material. The highest methane yield was obtained from untreated needles (213 mL CH_4/g VS) followed by pretreated bark (at the higher temperature), which resulted in 107 mL CH_4/g VS. Branches and cones yielded 98 and 75 mL CH_4/g VS after pretreatment at the lower temperature. Finally, alkaline pretreatment (8% w/w NaOH) at 100 °C for 10 min resulted in a methane yield of 178 mL CH_4/g VS (Salehian *et al.* 2013).

Ionic liquids have also been employed for the pretreatment of pine. More specifically, Shafiei *et al.* (2014) evaluated pretreatment with 85% NMMO at 120 °C for 30 min. Use of this pretreatment process resulted in improvement of the methane yield of pine chips from 21 mL/g VS to approximately 143 mL/g VS, whereas the methane yield from pine powder improved from 66 mL CH_4/g VS to approximately 224 mL CH_4/g VS.

Finally, organosolv pretreatment was employed as the pretreatment process by Mirmohamadsadeghi *et al.* (2014). The pretreatment was done with 75% ethanol supplemented with 1% w/w sulfuric acid, and it took place at 150 °C for 30 min. The methane yield obtained during solid-state digestion was 71 mL CH_4/g carbohydrates (which is equal to 54 mL CH_4/g VS).

Cedar

Japanese cedar (*Cryptomeria japonica*) is an endemic species in Japan. Take *et al.* (2006) used Japanese cedar in their work to evaluate the effects of a variety of pretreatment processes on the methane yield (refiner, steaming, biological with Basidiomycetes, and steam explosion). No methane was observed from the digestion of untreated material; whereas the refiner pretreatment alone resulted in only 8 mL methane (it was not clear if this was mL of total methane produced or methane yield). When refiner pretreatment was repeated 10 times, the methane increased to 26 mL. Pretreatment, when done with steaming alone (170 °C for 30 min), gave 28 mL methane, whereas when steaming was coupled to refining, the amount of methane obtained increased to 45 mL. Steam explosion was superior, resulting in up to 180 mL methane when the treatment took place at 258 °C for 5 min. Finally, of the different Basidiomycetes employed during this work, the most efficient one was *Cyathus stercoreus* AW 03-72, and the methane obtained reached 43 mL (Table 1).

Biological pretreatment of cedar chips was also evaluated by Amirta *et al.* (2006) by using white-rot fungi. More specifically, they used three strains of *Ceriporiopsis subvermispora* and *Pleurocybella porrigens* with the presence or absence of wheat bran for 4 to 8 weeks. The strain of *C. subvermispora* was more efficient for the pretreatment of cedar, with the maximum methane yield being obtained when the strain *C. subvermispora* ATCC 90467 was used for eight weeks in the presence of wheat bran. Under these conditions, the methane yield reached approximately 25% of the maximum theoretical yield, based on the holocellulose content of the original wood chips (there were no data available for the yield in mL CH_4/g VS).

Mixtures of Softwood

Mixtures of spruce and pine are rather common in the literature, as these two softwood species are not often separated in the timber industry. The most common pretreatment for mixtures of softwood in the literature has been with ionic liquids. For example, Kabir *et al.* (2013) evaluated the use of a mixture of spruce, pine, bark, *etc.*, for

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methane production. In the first stage, they evaluated the effect of the size of the materials (2, 4, and 8 mm). Although the methane yields of the untreated materials were low, it was found that the size of the material plays a very important role, with the 8-mm particles giving 0 mL CH_4/g VS and the 2-mm particles giving 70 mL CH_4/g VS. In the next stage, the effect of pretreatment with ionic liquids was evaluated using 75% to 85% w/w NMMO at 120 °C for 3 to 15 h. The best results were obtained when the material was treated with 85% NMMO for 15 h, resulting in 170 mL CH_4/g VS (Table 1). Finally, the authors evaluated the inhibitory effect of NMMO on anaerobic digestion, and demonstrated that even 0.0016% NMMO can result in a 34% reduction in methane yield. This is a very interesting observation, which underpins the importance of removing the NMMO from the pretreated materials before digestion.

In another study, Kabir *et al.* (2014) pretreated a softwood mixture with 85% NMMO at 90 °C for 30 h, and obtained a methane yield of 150 mL CH_4/g VS. They also evaluated the possibility of reusing the NMMO to reduce the cost of the process. Although they found that this was possible when they used barley straw as raw material; reusing NMMO for pretreatment of forest residues resulted in a decrease in methane yield. In an attempt to find the reason behind this effect, they measured the crystallinity of the pretreated material (both with fresh and recycled NMMO). It was found that when NMMO was reused, the crystallinity was higher than when they used fresh NMMO, and hence concluded that the NMMO lost its dissolution power with reuse. Finally, another combination of temperature and holding time (120 °C for 3 h) was evaluated by Aslanzadeh *et al.* (2014) when using 85% NMMO. They obtained 109 mL CH_4/g VS, which was higher than the yield from untreated material (42 mL CH_4/g VS). In the same study, a semi-continuous system was also evaluated for a mixture of spruce and pine, both untreated and pretreated. In both materials, the hydraulic retention time (HRT) was 20 days and the maximum organic loading rate (OLR) was 4.4 g VS/L/day. The daily productivity of the untreated material was 53 mL CH_4/g VS/day and for the pretreated material it was 92 mL CH_4/g VS/day.

Finally, a mixture of spruce, pine, bark, *etc.*, was also evaluated as raw material for anaerobic digestion by Kabir *et al.* (2015), using organosolv pretreatment. Untreated materials resulted in only 50 mL CH_4/g VS, whereas materials pretreated at 190 °C for 1 h improved the yield to the range 230 to 340 mL CH_4/g VS. Among the different solvents (50% v/v of either ethanol or methanol) and catalysts (sulfuric acid, acetic acid, or no catalyst) used, the two optimal combinations were ethanol with 1% w/w sulfuric acid and methanol with 1% w/w sulfuric acid. Both combinations resulted in 340 mL CH_4/g VS. From these two pretreatment methods, the authors concluded after conducting a techno-economic analysis of the process that use of methanol is more viable financially.

HARDWOODS

Birch

Birch (*e.g. Betula pendula* and *B. pubescens*) is a hardwood that grows worldwide and is very abundant in the northern part of Europe (Goshadrou *et al.* 2013; Vivekanand *et al.* 2013). Methane yield from untreated birch is generally higher than those from other untreated wood materials, and it can reach up to 250 mL CH_4/g VS (Mirahmadi *et al.* 2010), although much lower values (*e.g.*, 18 mL CH_4/g VS) have also been demonstrated in the literature (Matsakas *et al.* 2015).

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Different sorts of pretreatment techniques have been examined in order to improve the methane yield. Vivekanand *et al.* (2013) evaluated the effect of different operating conditions of steam explosion on the digestibility of birch. The highest methane yield that they obtained was 369 mL CH_4/g VS from birch pretreated at 220 °C for 10 min (Table 2). Moreover, they found that there was a correlation between the glucose released from enzymatic treatment and the methane yield obtained during anaerobic digestion. On the other hand, hydrothermal pretreatment using SO_2 as catalyst was found by us lead to a slightly lower methane yield of 305 mL CH_4/g VS (Matsakas *et al.* 2015). To obtain this yield, cellulolytic enzymes at a concentration corresponding to 15 FPU/g were added.

Apart from the thermal treatments, alkaline pretreatment at lower temperatures has also been evaluated. The alkaline pretreatment has proven to be more beneficial, as Mirahmadi *et al.* (2010) achieved higher methane yield (460 mL CH_4/g VS) when birch was treated with 7% w/w NaOH for 2 h at 100 °C. On the other hand, when NaOH was supplemented with 5.5% w/w thiourea and treated at −15 °C for 16 h, the methane yield was reduced to 360 mL CH_4/g VS (Mohsenzadeh *et al.* 2012).

Finally, another research group has also tried to evaluate ionic liquid pretreatment using NMMO (Goshadrou *et al.* 2013). Pretreatment with NMMO at 130 °C for 3 h resulted in an increase in methane production from 158 mL CH_4/g VS to 232 mL CH_4/g VS.

Willow

Willow (*Salix* spp.) is also an important genus that can be found in several countries around the world. Turick *et al.* (1991) evaluated different willow species without using any sort of pretreatment, except size reduction. The highest methane yield that they demonstrated reached 310 mL CH_4/g VS when either *S. eriocephala* or *S. exigua* was used (Table 2).

Horn *et al.* (2011) used the species *Salix viminalis* in their work. They evaluated different combinations of temperature and holding time during steam explosion pretreatment, by varying the temperature from 170 to 230 °C, whereas the holding time was set at 5, 10, or 15 min. Methane production after 57 days was more or less similar for a range of combinations of temperature and holding time, with the highest yield obtained being about 240 mL CH_4/g VS. Moreover, the authors found a correlation between the methane (and biogas) yield and the sugar release after enzymatic saccharification of the pretreated materials. As a result of this good correlation, they proposed that this could serve as a fast screening of the digestibility of lignocellulosic materials. In another study, Estevez *et al.* (2012) evaluated different operational conditions of steam explosion pretreatment of *Salix*, where the highest amount of methane was obtained with the combinations 230 °C for 5 min (241 mL CH_4/g VS), 230 °C for 10 min (234 mL CH_4/g VS), and 210 °C for 10 min (230 mL CH_4/g VS). In the next stage of their work, they also evaluated co-digestion of the pretreated *Salix* with cow manure at different ratios, which resulted in different carbon to nitrogen (C:N) ratios. For these experiments, they choose to use the pretreatment conditions of 210 °C for 10 min, as the improvement in methane yield observed under the harsher pretreatment conditions was not statistically significant. From the different mixtures that were evaluated, the highest methane yields were obtained for 30% *Salix* (228 mL CH_4/g VS) and 40% *Salix* (224 mL CH_4/g VS) in terms of total VS, which was equivalent to a C:N ratio of 35 and 39, respectively.

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Table 2. Different Sources of Hardwood Forest Materials and Yield Obtained during Anaerobic Digestion

Substrate	Pretreatment	Enzymes	Type of digestion	Methane yield (mL CH_4/g VS)	Reference
Birch	Steam explosion	-	Mesophilic	369	Vivekanand *et al.* 2013
Birch	Hydrothermal with SO_2	15 FPU/g cellulolytic	Thermophilic	305	Matsakas *et al.* 2015
Birch	Alkali	-	Thermophilic	460	Mirahmadi *et al.* 2010
Birch	Alkali/thiouria	-	Thermophilic	360	Mohsenzadeh *et al.* 2012
Birch	Ionic liquids	-	Thermophilic	232	Goshadrou *et al.* 2013
Willow	-	-	Mesophilic	310	Turick *et al.* 1991
Willow	Steam explosion	-	Mesophilic	240	Horn *et al.* 2011
Willow	Steam explosion	-	Mesophilic	241	Estevez *et al.* 2012
Willow and cow manure	Steam explosion	-	Mesophilic	228	Estevez *et al.* 2012
Willow	Aqueous ammonia soaking	-	Mesophilic	155	Jurado *et al.* 2013
Eucalyptus	Hot water extraction	-	Mesophilic	124[*]	Nakamura and Mtui 2003
Eucalyptus	Alkaline	-	Mesophilic	134[*]	Nakamura and Mtui 2003
Eucalyptus	Steam explosion	-	Mesophilic	194[*]	Nakamura and Mtui 2003
Poplar	-	-	Mesophilic	290	Turick *et al.* 1991
Poplar	Alkaline	-	Mesophilic	272	Yao *et al.* 2013
Elm	Organosolv	-	Mesophilic	75	Mirmohamadsadeghi *et al.* 2014
Japanese beech	Soxhlet extraction and supercritical water	-	Thermophilic	15.8[**]	Yoshida *et al.* 2010
Sycamore	-	-	Mesophilic	320	Turick *et al.* 1991
Black locust	-	-	Mesophilic	240	Turick *et al.* 1991
Sweetgum	-	-	Mesophilic	210	Turick *et al.* 1991
Maple	-	-	Mesophilic	57	Brown *et al.* 2012

[*]The results are mL CH_4/g TS and they were calculated by dividing the amount of methane (in mL) by the amount of dry chips added in the digestion (in g).
[**]Given as mL methane and not as yield.

Finally, soaking in aqueous ammonia was examined by Jurado *et al.* (2013) for the pretreatment of willow prior to digestion. Untreated willow resulted in 80 mL CH_4/g VS, whereas when pretreatment took place with 32% w/w ammonia solution at 25 °C for three days, the methane yield increased to 155 mL CH_4/g VS.

Eucalyptus

Eucalyptus is a tree genus that predominates in Australian forests. Apart from Australia, eucalyptus can be found in many other countries such as Brazil. Nakamura and Mtui (2003) used wood chips from *Eucalyptus globulus* and evaluated the following pretreatment processes: extraction with hot water (125 °C for 20 min), extraction in the presence of 1% NaOH (125 °C for 20 min), and steam explosion (25 atm for 3 min). Untreated eucalyptus chips gave very poor methane production of only 48 mL (which is equivalent to 14 mL CH_4/g TS (total solids) if one takes into consideration the amount of dry chips that the authors used). The hot water extraction was more efficient, resulting in 421 mL of methane (which is equivalent to 124 mL CH_4/g TS), whereas when supplemented with 1% NaOH the methane yield increased to 456 mL (134 mL CH_4/g TS). Finally, the highest methane production observed was when steam explosion was used, and it reached 660 mL (194 mL CH_4/g TS) (Table 2).

Poplar

Different species of poplar were evaluated by Turick *et al.* (1991) without previous pretreatment, except size reduction. The highest methane yield was obtained from the hybrid *Populus nigra x Populus maximowiczii* and it reached 290 mL CH_4/g VS (Table 2). Lower methane yield from untreated poplar was obtained by Yao *et al.* (2013), reaching a value of 127 mL CH_4/g VS. On the other hand, the same authors evaluated the use of alkaline pretreatment with various doses of NaOH at ambient temperature for 4 days. The moisture content was approximately 88%. Apart from the different concentrations of NaOH, they examined the effect of increasing the solid concentration in the digesters (from 35 to 80 g/L). The highest methane yield of 272 mLCH_4/gVS was obtained using 5% NaOH with initial solid content of 35 g/L. The authors observed a decrease in yield with increase in substrate content from 50 to 80 g/L. Finally, they mentioned that the decrease in methane yield at 80 g/L may have been caused by a combination of acidification and high levels of sodium ions.

Other Hardwoods

Mirmohamadsadeghi *et al.* (2014) examined the possibility of using elm trees as raw material for biogas production. The methane yield of the untreated material was low, reaching only 54 mL CH_4/g carbohydrates (which is equivalent to approximately 42 mL CH_4/g VS). When organosolv treatment was used (75% ethanol, 180 °C for 1 h), the methane yield improved to 94 mL CH_4/g carbohydrates (equivalent to approximately 75 mL CH_4/g VS). Although there was an improvement in yield, it still remained quite low.

Yoshida *et al.* (2010) used Japanese beech (*Fagus crenata*) as raw material for anaerobic digestion. They applied a two-step pretreatment. In the first stage, a Soxhlet extraction with 1:2 v/v ethanol:benzene took place for 12 h. In the second stage, supercritical water treatment was evaluated at 380 °C, where the pressure was either 30 or 100 MPa and the resistance time was 7, 60, or 240 s. According to their findings, a pressure of 30 MPa was more efficient than 100 MPa for all the holding times used. The highest methane production was observed when the treatment took place for 240 s at 30 MPa (15.8 mL methane, which corresponds to 117% of the maximum calculated yield). There were no available data concerning the methane yield per gram of VS or TS.

Turick *et al.* (1991) evaluated different hardwoods without any sort of pretreatment, except size reduction. During their work, untreated sycamore (*Platanus occidentalis*) gave a methane yield of 320 mLCH_4/g VS, whereas the yields from black locust (*Robinia*

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pseudoacacia) and sweetgum (*Liquidambar styraciflua*) were 240 mL CH_4/g VS and 210 mL CH_4/g VS, respectively. Finally, Brown *et al.* (2012) also evaluated untreated hardwood—more specifically, maple—to serve as raw material for both liquid and solid-state anaerobic digestion. Liquid anaerobic digestion was more efficient, with a methane yield of 57 mL CH_4/g VS, whereas the yield from solid-state anaerobic digestion was 47 mL CH_4/g VS.

CONCLUSIONS

Use of forest materials as raw material for anaerobic digestion has attracted much research interest during the last years. Forest materials are generally more difficult to be digested compared to other residues (*e.g.* agricultural residues) and require more harsh pretreatment processes. Different pretreatment techniques have been examined in the literature, such as steam explosion, hydrothermal, and alkaline. Their efficiency has been found to depend strongly on the source of the raw materials used. Although it is very difficult to conclude which pretreatment method is more appropriate (as it is also difficult to compare results between different works), there is a trend indicating that thermal treatments with sulfur dioxide and organosolv are the most appropriate for the pretreatment of softwoods. On the other hand, for hardwoods the trend is that the alkaline pretreatment was generally more beneficial, followed by hydrothermal and steam explosion. Moreover, although not commonly used, it was also shown that application of enzymes can also have a positive impact on methane yields. Further research in the field of enzyme application should be carried out in the future in order to improve the methane yields. As a general conclusion, it is shown that forest materials can be efficiently used for anaerobic digestion in lab scale. Of course further work should be conducted in order to increase even more the efficiency of this conversion.

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