

**LIGNOCELLULOSIC BIOREFINERY FOR WASTE-FREE
MANUFACTURING OF PHYTOESTROGENS BELONGING
TO LIGNANS, SUGARS FOR PRODUCTION OF ETHANOL
AND GROWING MEDIUM**

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(RECEIVED OCTOBER 2013)

ABSTRACT

Biorefineries in simplified definition are ways of waste-free and complex utilization of biomass for the production of usable products. Lignocellulosic biorefinery is processed as an example; knots up of coniferous wood are used as a source of lignans phytoestrogens. Phytoestrogens are nutraceuticals, which are important in the prevention of lifestyle diseases, cancer and osteoporosis. The high content of lignans is also found in flax seeds. This paper describes a method of isolating lignans from wood mass and waste processing from this production by means of acid hydrolysis into fermentable solution for the purpose of ethanol production. The results of this work introduce further processing of other waste by pyrolysis and activation of steam and CO₂ mixture on material with minimal salinity and satisfactory ion exchange capacity, which can be used as a substrate for sensitive cultures (substitution of clay and peat mixture).

KEYWORDS: Lignocellulosic biorefinery, lignans, hydrolysis, growing media, separated substance of digestates.

INTRODUCTION

The rapid decline of fossil fuels including diesel led to the concept of biorefineries, modern industrial plants processing renewable sources of energy in a waste-free way - biomass, waste biomass if possible, which is processed into three product groups: bio-energy, bio-fuels (gas,

liquid, solid) and chemicals from biomass (Kamm et al. 2006a).

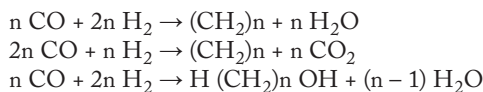
We distinguish the following biorefineries: Depending on the type of raw material and its main components, there are biorefineries processing lignocellulosic raw materials, raw sugar; green biorefineries process grasses, herbs and other lignocellulosic material with low lignin; other biorefineries process vegetable juices or grain. Biorefineries can also be divided by processes such as biorefineries of thermochemical processes, biorefineries of hybrids of thermochemical and biological processes (including enzymatic production). Moreover, we can classify biorefineries according to the orientation of production: fuel production, processing of agricultural waste, non-food products from cereals and many more. There are a number of often quite complicated definitions of biorefineries (NRC 2000, Kamm and Kamm 2004, Ohara 2003).

Although the current development of biorefineries is based on the latest technologies and conventional biotechnologies and the range of possible products is incredibly broad, their origins arose at the time of the development of chemical technology, focusing mainly on the processing of agricultural and forestry production (Kamm et al. 2006b).

Great development occurred in this area in the years 1933 - 1935 when the boom of hydrolysis of wood, straw and other cellulosic wastes began. The second wave of development comes in the years 1960 - 1972 (James 1969, Conrad 1962).

The development of biorefineries took another breath taking challenges to mining and diesel price in the world in connection with the development of underdeveloped countries civilization with large populations. The original Fischer-Tropsch synthesis was developed in detail; it was designed originally for the production of engine fuels, and many other substances from water gas ($\text{CO} + \text{H}_2$) produced by pyrolysis of coal-fed steam generator.

During the catalytic reaction by strongly exothermic reactions (Landa 1956):



there is a mixture of liquid and solid hydrocarbons; they are oxygenated compounds and gases. Hydrocarbons are alkanes or alkenes according to the conditions of synthesis.

Today, synthesis gases ($\text{CO} + \text{H}_2$) are produced by pyrolysis of biomass and composition of catalysts is a closely guarded secret. It is the real production of biodiesel. Today, biorefineries are able to produce of biomass a wide variety of various organic compounds for the pharmaceutical industry. They can produce thermoplastics and such substances that have never been found in biomass previously (Carlsson 1989).

Lignocellulosic biomass with a higher proportion of lignin, i.e. a biomass of trees, is the most important raw material for lignocellulosic biorefineries in the Czech Republic. The principle of processing lignocellulosic biomass in bio-refineries will probably be based mostly on its pyrolysis and subsequent processing of the resulting pyrolysis gases unlike lignocellulosic raw materials, i.e. biomass of grasses, herbs, cereals and other plants of agricultural production. There is relatively small proportion of lignin in these lignocellulosic raw materials. The main area of processing of this raw material in biorefineries will be hydrolytic processes, either chemical or enzymatic rather, with the following processing incurred carbohydrates.

Authors assume that lignin, hemicellulose and cellulose can be isolated of lignin biomass in biorefineries. (Kamm and Kamm 2004, Van Dyne 1999). Lignin is further processed into natural glues, adhesives, on bituminous coal and solid sulfur-free fuels. Hemicellulose will be a source of raw material for the production of xylose, xylitol and other substituted sugars and furfural. This

will be further processed into furan resin, chemical compounds of different nature and textile fibers of Nylon 6 and 6.6 types.

Further, hemicellulose will be processed into emulsifiers, adhesives, stabilizers and protective colloids for various industries. Cellulose will be used both ways: conventional way and partly by hydrolysis into glucose. We will continue to produce from glucose 5 - hydroxymethylfurfural, levulinic acid and further solvents, lubricants, chemicals and polymers. Another branch is processing of glucose by fermentation to ethanol, butanol, acetone, and organic acids, mainly lactic acid, for production of biodegradable polymers.

Large variety of lignocellulosic biorefinery products were described (Eaglesham et al. 2000, Kamm et al. 2006a, b). Our presented work represents a special form of lignocellulosic biorefinery. The main product of this experiment are valuable phytoestrogens, lignans (although flax seeds, belonging to functional foods, are known as a more accessible source of nutraceuticals). Other products are as follows: Sugar solutions (known technology) and new material derived from waste - growing medium for potted cultures. Kalač (2003) describes phytoestrogens and lignans including biological effects and Velíšek (1999) provides additional information.

Lignans are a broad group of plant phenols, which represents the structure of rich and diverse class of fenylpropanoid (C6 - C3) origin substances. Lignans can be divided into eight groups (Umezawa 2003, Harmatha 2005) according to the method of oxidation of propane units and their subsequent cyclization. In the late 19th century, these substances were identified in tree samples of different species and, therefore, in 1936, their name was proposed. As secondary metabolites of vascular plants the special effects have been found in them: antioxidant, antiviral, anticancer, antibacterial, oestrogenic, anti-oestrogenic, fungicidal, insecticidal and protective effect against vascular and heart and diseases (Ayres and Loike 1990 Smeds et al. 2007). It is logical that these physiological effects give hope to use these substances in the manufacture of pharmaceuticals and functional foods. They could be produced as a by-product of industrial wood processing (Holmbom et al. 2003). Finnish scientists have found unusually large amounts of lignans (6-24 wt %) in knots of Norway spruce (*Picea abies*) and found that the dominant lignan (70-85 %) is 7-hydroxymatairesinol, called HMR (Marešová 2014, Holmbom et al. 2003, Willför et al. 2006). Knots contain up to 100 times the amount of lignans than heartwood of the tree (Willför et al. 2006). The highest content of lignans in food and raw materials was found in flax seeds, sesame seeds, blackberries and cranberries, lower concentrations were found in grains, vegetables, fruit, legumes, tea, coffee and wine as well (Nurmi et al. 2003, Willför et al. 2003, Smeds et al. 2012a, Li et al. 2008, Marešová 2014).

MATERIAL AND METHODS

Complex and extensive problems of extraction, separation and characterization of lignans, their methods are dependent on the matrix, ie source material, is treated in detail in a doctoral dissertation under the guidance of prof. Ing. Jan Tríska, PhD., by co-authors of this study (Marešová 2014). Because lignans in wood matter occur in a free form of aglycones, the direct extraction with an aqueous ethanol solution or even water can be used to isolate them. Methodology for the extraction of knots from wood mass is protected by a utility model (Strohalm et al. 2012). Wood mass of knots after the disintegration on the cutting mill is first extracted - by means of hexane to remove lipophilic substances. Then the material is extracted with a mixture of acetone and water in a ratio of 70:30. Mixed acetone extracts are evaporated in a rotary vacuum evaporator and dissolved in ethanol. Ethanol solution is saturated at 50°C with potassium acetate

(AcOK) and stored in peace at 6°C when adduct HMR and acetate precipitate. The crystals of the adduct of HMR-AcOK are washed by a saturated solution of potassium acetate in ethanol and the adduct is extracted with a mixture of dichloromethane and water in the ratio 1:1. The proportion of methylene chloride is separated and evaporated in a rotary vacuum evaporator (Freudenberg and Knof 1957, Smeds et al. 2012a, b, Marešová 2014).

We hydrolysed waste wood by acid process, according to Harris and Beglinger (1946), based on Scholler's technology (1923 and 1935) after Katzen's modification (2000), although there are more appropriate methods to use (James 1969). Processes of total hydrolysis with dilute acid are already used in practice for the production of ethanol from biomass (Stone and Webster 1982). Hydrolysis products are not further followed because the whole hydrolysis process is generally known. We wondered what wastes from the production, we tried to be further processed by enzymatic hydrolysis of microorganisms of the *Clostridium* genus (Matsui 1991). Experiments were unsuccessful, although we have previously modified material in the process STEAM-EXPLOSION, ie rapid decompression by pressurized steam of heated material. Therefore, we have established the results of expired patents of IG Farbenindustrie of the forties of the last century, which aimed to produce cheap ion exchangers, oxidation processes, dehydration and sulfonation of coal waste sludges and organic pyrolysed wastes (IG Farbenindustrie AG, 1942, 1944). These ion exchangers, that time, did not work well because they had poor physical and mechanical properties; the particles disintegrated and clogged filters. However, it would be possible to make use of well materials in manufacturing of substrates for the cultivation of seedlings if there is advantageous environment with a low salt content, while a higher ion exchange capacity. Selected peat is commonly used for this purpose and testing biogas plants digestate is also used for the same purpose (Dubský et al. 2012).

Wastes after acid hydrolysis of wood mass were subjected to pyrolysis and activation of a mixture of steam + CO₂ (Drožalina and Barančíková 1969) and sulphonation described by Batt was performed then (2012). The product had an ion exchange capacity depending on reaction conditions of the initial composition of wood wastes in the interval 220-300 mmol.chem.eqv. H⁺1000⁻¹ g, which is very small value for industrial ion exchange but for making growing substrate it is sufficient and comparable to that used by a mixture of clay and peat. Sulfonation caused only 5-20 % increase in the ion-exchange capacity and therefore we completely retreated from the application of sulfonation due to complications in the manufacturing process. By saccharification process of wood mass, we came to a solution of 2.1 % of fermentable sugars, which is only about a half of normal results in an industrial facility for wood mass hydrolysis. However, we focused on hydrolysis wastes and therefore we have not followed the process of saccharification.

Pyrolysis of the waste after acid hydrolysis of wood was performed at 400 - 900°C, and the activation was carried out by means of a mixture of saturated water vapor and CO₂ in a volume ratio of 2:1 at a temperature of 100 - 110°C (Drožalina and Barančíková 1969). As the effects of pyrolysis temperature on the properties of the product were indistinct, later experiments have been carried out only in the temperature range 500 - 600°C.

Since the processed waste should replace peat during substrate production with a share of 20 % of separator from agricultural biogas plants processing a substrate mixture of pig slurry, maize silage and grass silage, we monitored separates of various biogas plants, peat and mixtures: separate + peat, separate + pyrolyzed and activated wood waste after acid hydrolysis.

RESULTS

Results showed that the most simple and adequately effective way in lignocellulosic biorefinery - for waste-free wood utilizing from knotty coniferous material - is normal acid hydrolysis with STEAM EXPLOSION pretreatment. The goal is to obtain saccharide solution for fermentative production of ethanol and wastes processing from hydrolysis by pyrolysis at 500 - 600°C, and activating the product by mixture of steam and CO₂ by Drožalina and Barančíková (1969). The product can then completely replace peat in the mixing process with 20 % addition of dried separator of agricultural biogas plants.

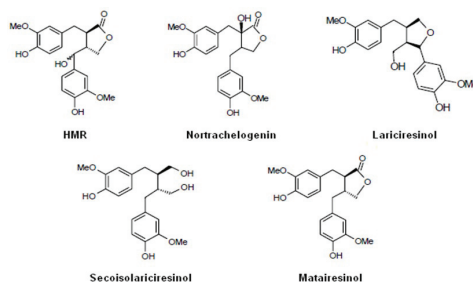


Fig. 1: Major lignans contained in soft wood species.

Tab. 1: Chemical and physical properties of separates of the South Bohemia agricultural biogas plants.

Separate BPS	VW wet sample	VW dry sample	Dry matter	Conductivity	N-NH ₄	N-NO ₃	K ⁺	CEC	AC	EAW
	g l ⁻¹	g l ⁻¹	%	mS.cm ⁻¹	mg l ⁻¹	mg l ⁻¹	mg J ⁻¹	mmol. chem.equiv. H ⁺ 1000 ⁻¹ g	% vol.	% vol.
Hosín	480	72	23	1.20	592	19	963	65	53	17
Jarošovice	324	64	20	1.19	684	21	815	58	61	12
Novosedly	510	80	24	1.31	703	26	1100	74	50	17

BPS – biogas plants, VW – volume weight, CEC – cation exchange capacity, AC – air capacity, EAW – easily available water

Tab. 2: Chemical and physical properties of the mixture A, mixture B, and peat itself Hospráz.

Separate BPS	VW wet sample	VW dry sample	Dry matter	Conductivity	N-NH ₄	N-NO ₃	K ⁺	CEC	AC	EAW
	g l ⁻¹	g l ⁻¹	%	mS.cm ⁻¹	mg l ⁻¹	mg l ⁻¹	mg J ⁻¹	mmol. chem.equiv. H ⁺ 1000 ⁻¹ g	% vol	% vol
mixture A	-	104	-	0.35	96	3.2	208	196	24.2	26.4
mixture B	-	118	-	0.44	102	3.1	195	138	29.7	22.1
peat HOSPRÁZ	580	136	23.4	0.12	0.1	0.0	12	238	8.3	30.2

mixture A 20 % dry separate and 80 % peat HOSPRÁZ, mixture B 20 % dry separate and 80 % waste after acid hydrolysis of wood, pyrolyzed and activated separate according to Drožalina and Barančíková 1969

BPS – biogas plants, VW – volume weight, CEC – cation exchange capacity, AC – air capacity, EAW – easily available water

Growing media for potted cultures, excluding corresponding physical properties ensuring the optimal balance between water and air for the young plants, must have an adequate amount of available plant nutrients. The separated substance of digestate from biogas plants is a cheap source of these nutrients, but too rich, especially in terms of the content of ammonium nitrogen and potassium accessible. Therefore the separate is mixed with peat, which reduces the concentration of these nutrients as well as the salt content (expressed by conductivity) and the pH at the desired level. Yet it is necessary dry the separate previously because drying separator reduces the amount of N-NH_4^+ for about a fifth of the amount, which fresh separate includes. The optimum content of N-NH_4^+ in the substrate is about 100-120 mg.l^{-1} and the content of K^+ is about 200-300 mg.l^{-1} . The conductivity of aqueous extract 1V/5V is optimal within 0.3 - 0.4 mS.cm^{-1} and pH is in the range 5.5 - 6.5. The separate itself is alkaline, the acidic peat neutralizes it.

Commonly used peat substrates have air capacity of about 13 vol %, easily available water represents the difference in water content under negative pressure 10 and 50 cm around 32 %. The separate addition increases the size of the air separator capacity and reduces the amount of easily reachable water.

Tab. 2 shows that the processed wood waste according to the present method is very close to its properties of peat sites Hospříz and growing media with 20 % addition of the powder separator from agricultural biogas with 80 % of the processed wood waste in comparison with substrates of the separator and peat are very similar. Therefore peat can be completely replaced by this product in the production of substrate. Opposed peat, the substrate of this waste has a relatively lower ion exchange capacity CEC.

Separates of individual agricultural biogas plants are due to virtually the same fermented substrate (slurry + corn silage, grass silage + grass haylage) virtually identical to the required criteria.

Relatively high content of lignans from HMR among other lignans in spruce knots, relatively easy opportunity of extraction compared with the isolation of lignans in other sources of biomass than the wood mass and the possibility of full utilization of waste to replace peat in growing media represent a prerequisite for the success of this type of biorefinery and for isolation of technological experiments. Just operating results, including technology-intensive analytical control together with interests of manufacturers who produce pharmaceuticals and functional foods will determine the profitability of biorefineries. The economic aspect of replacement of peat by processed production waste is necessary to be assessed from an environmental perspective. Today, peat is imported to the Czech Republic relatively cheaply and in any amount from Belarus and the Baltic Republics. The question is how long these countries let their peat resources and landscape devastate by peat mining for export.

Our hopes for the application of sulfonation were not fulfilled. The average increase of achieved value expressing the ion exchange capacity T was only 10-12 % in the range 5-20 % and thus we watched the cause of the failure. So we repeated experiments of mentioned patents of IG Farbenindustrie from the last century, and we found that when using caustobioliths as basic materials it is possible to achieve even better results than it was reported by careful German authors. When using hydrolyzed or pyrolyzed organic wastes, which are presented by patents' authors as another possible alternative material without reporting the results, however, in that case, the same way of sulfonation substantially fails. We explain it as follows: Caustobioliths have in their mass greater or lesser amounts of humus acids, ie humic and fulvic acids, or at least their chemical precursors. Cores of these acids are aromatic and readily undergo sulfonation. Wood wastes, that we modified via hydrolysis and pyrolysis, have an active surface but there are no or less aromatics in these materials. Sulfonation is then just a random and inefficient. This

has been confirmed by the results of Batt (2012), who used hydrolysed kitchen wastes modified during sulfonation by means of four methods (oleum, sulfur oxide, sulfur dioxide, oxygen, and nitrogen oxides, sulfur dioxide and chlorine, followed by alkaline hydrolysis) and values of T were only 33–69 mmol of chemical equivalent of hydrogen /1000 g. This corresponds to our findings concerning sulfonation of hydrolyzed and pyrolyzed wood wastes without adjusting according Drožalina and Barančíková (1969). We reached maximum value of T = 60 mmol of chemical equivalent of hydrogen /1000 g. On the basis of existing professional literature focused on biorefineries (Kamm et al. 2006a, b, Ohara 2003, Van Dyne 1999, Eaglesham et al. 2000) it is evident, that cellulose and lignin growing media have not been studied yet, during the complex processing of biomass. Attention in the bio-refineries of this type is directed to the processes of hydrolysis and processing incurred saccharide solutions and above all to the processes of pyrolysis and processing of pyrolysed gases that are variously modified by catalytic Fischer-Tropsch synthesis with resulting hydrocarbons; these are mainly alkanes. The main challenge is then real production of biodiesel, for which it is now commonly regarded a vegetable oil processed by methylation.

DISCUSSION

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CONCLUSIONS

Our hopes for the application of sulfonation were not fulfilled. The average increase of achieved value expressing the ion exchange capacity was only 10-12 % in the range 5-20 %. So we repeated experiments of mentioned patents of IG Farbenindustrie from the last century, and we found that when using caustobioliths as basic materials it is possible to achieve even better results than it was reported by careful German authors. When using hydrolyzed or pyrolyzed organic wastes, which are presented by patents' authors as another possible alternative material without reporting the results, however, in that case, the same way of sulfonation substantially fails. We explain it as follows: Cores of humic and fulvic acids are aromatic and readily undergo sulfonation. Wood wastes that we modified via hydrolysis and pyrolysis have an active surface but there are no or less aromatics in these materials. Sulfonation is then just a random and inefficient. This has been confirmed by the results of Batt (2012), who used hydrolysed kitchen wastes modified during sulfonation by means of four methods (oleum, sulfur oxide, sulfur dioxide, oxygen, and nitrogen oxides, sulfur dioxide and chlorine, followed by alkaline hydrolysis) and values of T were only 33-69 mmol of chemical equivalent of hydrogen / 1000 g. This corresponds to our findings concerning sulfonation of hydrolyzed and pyrolyzed wood wastes without adjusting according to Drožalína and Barančíková (1969). We reached maximum value of T = 60 mmol of chemical equivalent of hydrogen / 1000 g. On the basis of existing literature focused on biorefineries (Kamm et al. 2006a, b, Ohara 2003, Van Dyne 1999, Eaglesham et al. 2000) it is evident, that cellulose and lignin growing media have not been studied yet, during the complex processing of biomass. Attention in the bio-refineries of this type is directed to the processes of hydrolysis and processing incurred saccharide solutions and above all to the processes of pyrolysis and processing of pyrolysed gases that are variously modified by catalytic Fischer-Tropsch synthesis with resulting hydrocarbons; these are mainly alkanes. The main challenge is then real production of biodiesel, for which it is now commonly regarded as vegetable oil processed by methylation.

ACKNOWLEDGMENT

The study was supported by Grant of Ministry of Agriculture of the CR (Project No. QJ1230066 and QI 91C200) and by Grant of Ministry of Education, Youth and Sports of the Czech Republic, grant number COST LD11016, and by CzechGlobe—Centre for Global Climate Change Impacts Studies, Reg. No. CZ.1.05/1.1.00/02.0073. We are very grateful for this support.

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