

## Monomeric phenolic compounds from hydrolyzed waste lignocellulosic materials

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Alkaline treatment (4 and 6 hours) of hydrolyzed waste lignocellulosic materials was carried out. Monomeric phenolic compounds: 2-methoxyphenol, 3-ethylphenol, 4-ethyl-2-methoxyphenol, 2,6-dimethoxyphenol, 4-hydroxy-3-methoxybenzaldehyde, 4-hydroxy-3,5-dimethoxybenzaldehyde, 1-(4-hydroxy-3-methoxyphenyl) ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl) ethanone, etc. were obtained. Toluene and ethyl acetate were used for extraction of the monomeric products.

**Keywords:** Waste hydrolyzed lignocellulosic materials; Alkaline treatment; Phenolic compounds; Extraction.

### INTRODUCTION

The chemical treatment of wood to produce cellulose and monosaccharides is accompanied by the production of about one third of its mass of lignin-rich residue. The production of bioethanol from raw materials has been increasing, and hence, the utilization of lignin has started to attract significant interest.

The alkaline hydrolysis of lignin results in its depolymerization through the rupture of ether and C-C bonds connecting the phenylpropane units. The method leads to the production of low-molecular weight monomeric phenols (2,6-dimethoxyphenol, 3,6-dimethoxy-4-hydroxy acetophenone, 4-ethylguaiacol, 2-methoxyphenol, 4-ethyl-2-methoxyphenol, 2-methoxy-4-propylphenol, 4-methylcatechol, phenol, 4-hydroxy-3,5-dimethoxybenzaldehyde, and 4-hydroxy-3-methoxybenzaldehyde [1]. Waste lignocellulosic biomass has been shown to be a suitable source of monomeric phenolic compounds [2]. The monomeric and oligomeric lignin-derived products have been produced by supercritical water treatment of softwood and hardwood [3-5] and might be useful for substituting the aromatic chemicals from fossil resources.

In our earlier investigations, the antioxidation effect of methoxyphenols produced from lignin was demonstrated by the oxygen absorption of liquid paraffin in a laboratory manometric installation [6] and by determining the gasoline induction period [7]. Furthermore, alkaline treatment is widely applied in hydrolyzed residual lignin activation aiming its utilization [8]. In the cited paper it was found that the treatment with 5.0 % NaOH leads to

the production of lignin products with the highest hydrophilicity and the best porosity.

The aim of the present study was to investigate the nature of the low-molecular products extracted with a polar and a non-polar solvent from the liquid phase after the alkaline treatment, from hydrolyzed waste lignocellulosic materials. Steam explosion and dilute acid hydrolysis were used as pretreatment methods. Their application was followed by enzymatic hydrolysis with cellulase enzyme complex combined with  $\beta$ -glucosidase [9].

### EXPERIMENTAL

The process conditions were determined based on the results of our earlier investigations [10, 11]. The determination of lignin was carried out according to TAPPI standard test method T 222 om-11.

The alkaline treatment of hydrolyzed waste lignocellulosic materials was carried out in stirred autoclaves made of stainless steel, heated in a bath of polyethylene glycol at 180 °C for 4 and 6 hours. An aqueous solution of NaOH (5%) was used as a depolymerization agent, at a ratio of waste materials to aqueous NaOH solution of 1:8. The principal scheme for the production of low-molecular compounds from hydrolyzed waste lignocellulosic materials is shown on Fig.1. The destructed lignin suspension obtained by alkaline treatment was filtered, and the quantity of solid residue was determined. The dissolved part was precipitated by acidification with HCl (2 mol/L) to pH 1 - 2. The volume of the liquid part separated by filtration was reduced by rota evaporation. A triple extraction was carried out at a toluene to water ratio of 1:5. The toluene extract was dried up with anhydrous Na<sub>2</sub>SO<sub>4</sub> and then filtered. The liquid phase separated after the toluene extraction was subjected to a double extraction with ethyl acetate at an organic to water ratio of 1:5. The amounts of obtained extracts,

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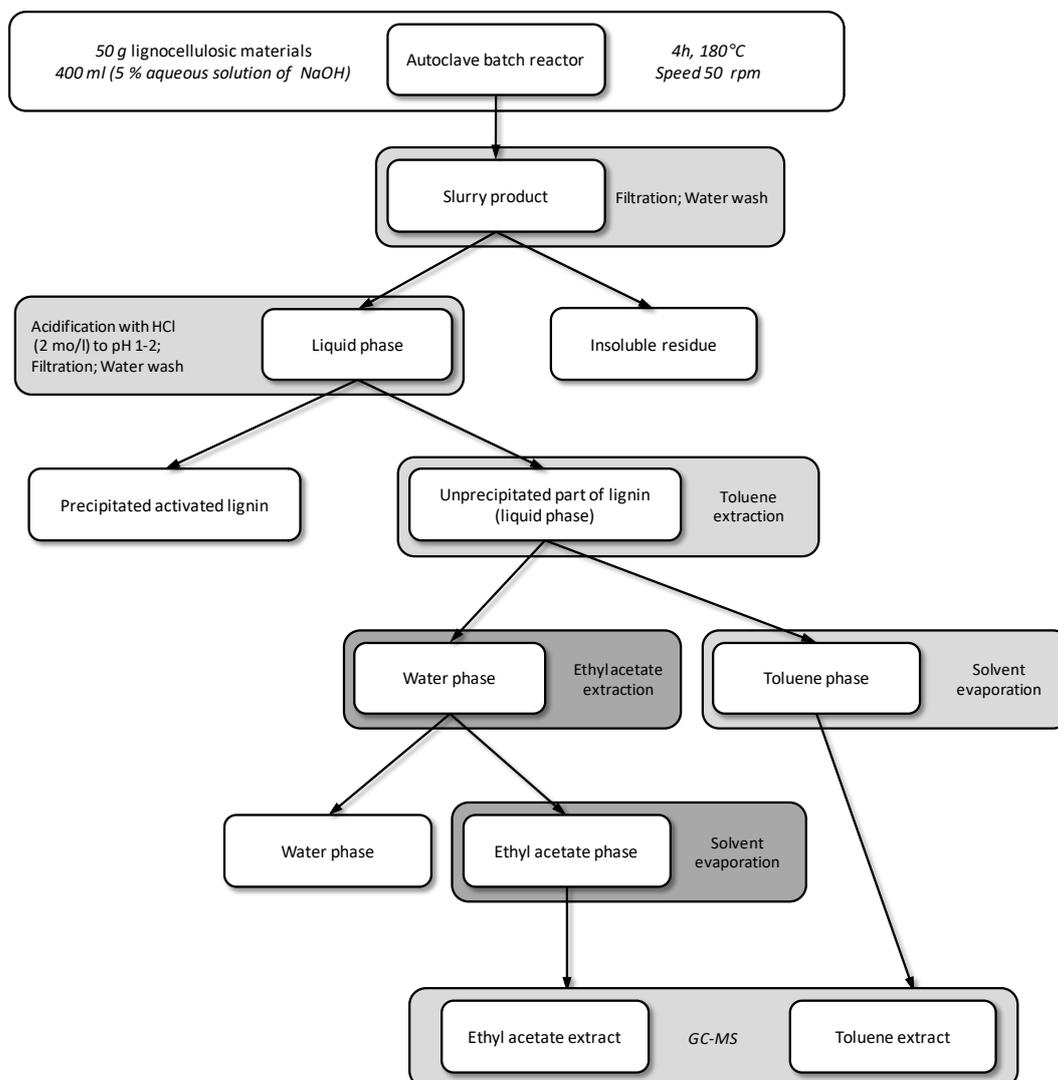
insoluble residue, precipitated and non-precipitated lignin were determined.

The compounds were identified by GC-MS analysis with a KONIK gas chromatography unit, model HRGC 5000B, equipped with a mass selective detector, MS Q 12. A 15 m × 0.25 mm i.d. capillary column KNK 5 with a film thickness of 0.25 μm was used. The GC oven temperature was held at 40 °C for 5 min and then programmed to attain a temperature of 270 °C at a 10 °C/min ramping rate. The injector temperature was 280 °C with 20:1 split ratio. Helium was used as the carrier gas at a constant flow rate of 1.3 mL/min. Typical mass spectrometry operating conditions were as follows: temperature of transfer line, 250 °C; temperature of ion source, 245 °C; electron energy, 70 eV.

## RESULTS AND DISCUSSION

The lignin content determined in the hydrolyzed waste lignocellulosic materials is shown in Table 1. The yield of products from the alkaline treatment of hydrolyzed waste lignocellulosic materials is shown in Table 2. During alkaline treatment, 55 to 75% of the starting material became soluble (Table 2). These values include both solubilized lignin and hydrolyzed polysaccharides. Some cellulose and lignin remained as insoluble residue. The main portion of lignin was destructed and dissolved in the alkaline solution. From the solution, after acidification, alkaline activated lignin was separated; the liquid phase contained low-molecular phenol compounds (LMPC) from the decomposed lignin.

It was found that by increasing the process duration, enhanced yield of monomeric compounds was obtained.



**Fig. 1.** Scheme for production of low-molecular compounds from hydrolyzed waste lignocellulosic materials.

**Table 1.** Lignin content in hydrolyzed waste lignocellulosic materials

| Waste materials from                                             | Lignin content [%] |
|------------------------------------------------------------------|--------------------|
| Acid (130°C) and enzymatic hydrolysis of wheat straw             | 40.2               |
| Steam explosion (190°C) and enzymatic hydrolysis of wheat straw  | 36.6               |
| Acid (130°C) and enzymatic hydrolysis of maize stalks            | 42.7               |
| Steam explosion (190°C) and enzymatic hydrolysis of maize stalks | 38.3               |
| Steam explosion (190°C) and enzymatic hydrolysis of paulownia    | 37.4               |

**Table 2.** Yield of products from the alkaline treatment of hydrolyzed waste lignocellulosic materials

| Waste materials                              | Duration of treatment [h] | Undissolved residue [%*] | Dissolved part           |                                               |                      |                            | Total yield of monomeric compounds [% of lignin content] |
|----------------------------------------------|---------------------------|--------------------------|--------------------------|-----------------------------------------------|----------------------|----------------------------|----------------------------------------------------------|
|                                              |                           |                          | Precipitated lignin [%*] | Unprecipitated part in the aqueous phase [%*] | Toluene extract [%*] | Ethyl acetate extract [%*] |                                                          |
| Wheat straw<br>Acid pretreatment             | 4                         | 29.64                    | 22.46                    | 47.90                                         | 1.64                 | 1.50                       | 7.81                                                     |
| Wheat straw<br>Steam explosion pretreatment  | 4                         | 44.53                    | 25.14                    | 30.33                                         | 1.88                 | 3.68                       | 15.19                                                    |
|                                              | 6                         | 28.75                    | 28.43                    | 42.82                                         | 3.00                 | 3.77                       | 18.50                                                    |
| Maize stalks<br>Acid pretreatment            | 4                         | 36.44                    | 16.48                    | 47.08                                         | 0.87                 | 2.13                       | 7.02                                                     |
|                                              | 6                         | 27.83                    | 26.49                    | 45.68                                         | 3.97                 | 3.30                       | 17.02                                                    |
| Maize stalks<br>Steam explosion pretreatment | 6                         | 29.26                    | 21.60                    | 49.14                                         | 3.27                 | 3.74                       | 18.28                                                    |
| Paulownia<br>Steam explosion pretreatment    | 6                         | 24.34                    | 19.40                    | 56.26                                         | 3.93                 | 2.22                       | 16.45                                                    |

Note: \* mass percentage of initial material.

With 4 h of alkaline treatment, the highest yield of low molecular compounds was obtained by steam explosion of enzymatic hydrolyzed straw (15.19% mass of lignin). The highest yield of soluble and precipitated lignin was obtained by this process. It was found that the total yield of low-molecular compounds with acid pretreatment is considerably lower than the yield obtained with steam explosion pretreatment. (7.81% mass of lignin for wheat straw and 7.02% mass of lignin for maize stalks). Probably, the acid pretreatment leads to some condensation in the chemical structure of the lignin.

With 6 h of alkaline treatment, the highest yield of low molecular compounds was obtained again by steam explosion of enzymatic hydrolyzed straw (18.50% mass of lignin). The yield obtained from steam explosion of maize stalks was 18.28%, followed by the yield from acid pretreatment of

maize stalks (17.02%) and the yield from steam explosion of paulownia (16.45 %).

Tables 3 and 4 present the low-molecular compounds and their contents identified in the toluene extracts and in the ethyl acetate extracts.

The following compounds were identified in both extracts, but in different quantities: 2-methoxyphenol, 3-ethylphenol, 4-ethyl-2-methoxyphenol, 2,6-dimethoxyphenol, 4-hydroxy-3-methoxybenzaldehyde, 4-hydroxy-3,5-dimethoxybenzaldehyde, 1-(4-hydroxy-3-methoxyphenyl) ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl) ethanone, etc. The polar solvent ethyl acetate extracts mainly carbonyl compounds, such as ketones, aldehydes, and carbonic acids, which are not extracted by toluene.

The main compounds extracted with toluene are: 2,6-dimethoxyphenol (from 13.7 to 46.4 Area %); 1-(4-hydroxy-3,5-dimethoxyphenyl) ethanone (from

**Table 3.** Compounds identified in the toluene extracts

| Compounds                                  | Retention time, [min] | Wheat straw (Acid) | Wheat straw (Steam explosion) |      | Maize stalks (Acid) |      | Maize stalks (Steam explosion) | Paulownia (Steam explosion) |
|--------------------------------------------|-----------------------|--------------------|-------------------------------|------|---------------------|------|--------------------------------|-----------------------------|
|                                            |                       | [Area,%]           | [Area,%]                      |      | [Area,%]            |      | [Area,%]                       | [Area,%]                    |
|                                            |                       | 4h                 | 4h                            | 6h   | 4h                  | 6h   | 6h                             | 6h                          |
| Unidentified compound                      | 5.20                  | 1.2                | 0.8                           | -    | -                   | -    | -                              | -                           |
| Benzaldehyde                               | 5.91                  | 9.1                | 2.9                           | -    | 3.4                 | -    | -                              | -                           |
| Phenol                                     | 6.71                  | 5.7                | 1.5                           | -    | 1.8                 | -    | -                              | -                           |
| Unidentified compound                      | 7.67                  | 8.9                | 4.2                           | -    | 4.7                 | -    | -                              | -                           |
| Unidentified compound                      | 7.80                  | 1.3                | -                             | -    | -                   | -    | -                              | -                           |
| Unidentified compound                      | 7.93                  | 1.0                | 1.0                           | -    | 3.2                 | -    | -                              | -                           |
| 1,3-cyclopetanedione                       | 8.33                  | 2.6                | 2.4                           | -    | 1.8                 | -    | 4.0                            | -                           |
| 2-methoxyphenol                            | 8.85                  | 22.8               | 24.2                          | 23.2 | 15.9                | 14.6 | 16.2                           | 11.6                        |
| Unidentified compound                      | 9.43                  | -                  | 1.2                           | -    | 1.2                 | 6.6  | 3.9                            | 11.7                        |
| 3-Ethylphenol                              | 9.92                  | 1.8                | 3.8                           | -    | 3.8                 | -    | -                              | -                           |
| 4-Ethyl-2-methoxyphenol                    | 11.91                 | -                  | 1.2                           | 10.8 | 1.7                 | 10.9 | 3.7                            | -                           |
| Unidentified compound                      | 11.96                 | -                  | -                             | -    | -                   | -    | 3.4                            | -                           |
| Unidentified compound                      | 12.01                 | -                  | -                             | -    | -                   | -    | 5.5                            | -                           |
| 2,6-Dimethoxyphenol                        | 12.89                 | 13.7               | 16.5                          | 29.2 | 23.2                | 29.8 | 40.6                           | 46.4                        |
| 4-Hydroxy-3-methoxybenzaldehyde            | 13.52                 | 2.9                | 9.1                           | -    | 2.7                 | -    | -                              | -                           |
| 1-(4-Hydroxy-3-methoxyphenyl) ethanone     | 14.62                 | 3.1                | 6.5                           | -    | 2.9                 | 4.3  | -                              | -                           |
| Unidentified compound                      | 15.12                 | -                  | 0.7                           | -    | -                   | -    | -                              | -                           |
| 4-Hydroxy-3,5-dimethoxybenzaldehyde        | 16.63                 | 1.1                | 1.4                           | -    | 1.8                 | 4.4  | -                              | -                           |
| 1-(4-Hydroxy-3,5-dimethoxyphenyl) ethanone | 17.43                 | 24.8               | 22.6                          | 36.8 | 27.1                | 29.4 | 22.7                           | 30.3                        |
| Unidentified compound                      | 17.76                 | -                  | -                             | -    | 1.1                 | -    | -                              | -                           |
| Unidentified compound                      | 20.23                 | -                  | -                             | -    | 2.6                 | -    | -                              | -                           |
| Unidentified compound                      | 24.39                 | -                  | -                             | -    | 1.1                 | -    | -                              | -                           |

22.6 to 36.8 Area %) and 2-methoxyphenol (from 11.6 to 24.2 Area %).

### CONCLUSIONS

By extraction with two solvents, the following lignin degradation products were isolated: 2-methoxyphenol, 3-ethylphenol, 4-ethyl-2-methoxyphenol, 2,6-dimethoxyphenol, 4-hydroxy-3-methoxybenzaldehyde, 4-hydroxy-3,5-dimethoxybenzaldehyde, 1-(4-hydroxy-3-methoxyphenyl)

ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl) ethanone, etc.

The total yield of extracted monomeric compounds after 6 h of alkaline treatment is from 16.45% mass of lignin (paulownia, steam explosion) to 18.50% mass of lignin (straw, steam explosion followed by enzymatic hydrolysis). It was found that the highest total yield of low-molecular compounds from the waste was obtained with steam explosion pretreatment. The acid pretreatment may lead to lignin condensation.

**Table 4.** Compounds identified in the ethyl acetate extracts

| Compounds                                   | Retention time, min | Wheat straw | Wheat straw (Steam explosion) |      | Maize stalks (Acid) |      | Maize stalks (Steam explosion) | Paulownia (Steam explosion) |
|---------------------------------------------|---------------------|-------------|-------------------------------|------|---------------------|------|--------------------------------|-----------------------------|
|                                             |                     | (Acid)      | [Area,%]                      |      | [Area,%]            |      | [Area,%]                       | [Area,%]                    |
|                                             |                     | 4h          | 4h                            | 6h   | 4h                  | 6h   | 6h                             | 6h                          |
| Unidentified compound                       | 4.00                | 5.2         | -                             | -    | 3.7                 | -    | -                              | -                           |
| Unidentified compound                       | 4.26                | 3.2         | -                             | -    | -                   | -    | -                              | -                           |
| Benzaldehyde                                | 5.84                | 1.3         | -                             | -    | 3.6                 | -    | -                              | -                           |
| Phenol                                      | 6.66                | 6.0         | 13.1                          | 14.5 | 7.0                 | -    | -                              | -                           |
| 1,2- Cyclopentanedione-3-methyl-            | 7.68                | 3.1         | -                             | -    | 1.6                 | -    | -                              | -                           |
| 1,3-Diethylbenzene                          | 8.04                | 1.4         | -                             | -    | 3.3                 | -    | -                              | -                           |
| 1,3-Cyclopentanedione 2,4-Dimethyl-         | 8.28                | 5.8         | 7.5                           | -    | 2.1                 | -    | 7.6                            | 4.6                         |
| 2-methoxyphenol                             | 8.82                | 8.9         | 8.5                           | 4.0  | 12.2                | 5.6  | 3.5                            | 17.3                        |
| Unidentified compound                       | 9.02                | 2.7         | 8.0                           | -    | 9.0                 | -    | -                              | 16.7                        |
| Unidentified compound                       | 9.40                | -           | -                             | -    | 1.2                 | -    | -                              | -                           |
| Unidentified compound                       | 9.64                | -           | 6.1                           | -    | 1.6                 | -    | -                              | 5.2                         |
| Unidentified compound                       | 9.92                | 1.0         | -                             | -    | 2.0                 | -    | -                              | -                           |
| Benzoic acid                                | 10.32               | 1.6         | -                             | -    | 2.0                 | -    | -                              | 6.4                         |
| Benzenediol                                 | 10.76               | 3.7         | -                             | -    | 3.0                 | -    | -                              | 10.8                        |
| 3-Methoxy-1,2-benzenediol                   | 11.62               | 3.3         | 6.0                           | -    | -                   | -    | -                              | 6.8                         |
| 2,6-Dimethoxyphenol                         | 12.89               | 10.2        | 7.9                           | 9.0  | 10.4                | 11.7 | 17.0                           | 13.1                        |
| 4-Hydroxybenzaldehyde                       | 13.04               | 1.7         | -                             | -    | -                   | -    | -                              | -                           |
| 4-Hydroxy-3-methoxybenzaldehyde             | 13.49               | 5.4         | 5.3                           | 25.6 | 3.2                 | 13.3 | 14.9                           | 4.7                         |
| 1-(2-Hydroxyphenyl) ethanone                | 14.05               | 2.3         | 4.9                           | -    | -                   | -    | -                              | -                           |
| 1-(4-Hydroxy-3-methoxyphenyl) ethanone      | 14.59               | 2.5         | -                             | 3.3  | 2.1                 | 8.6  | -                              | -                           |
| 4-Hydroxy-3-methoxybenzoic acid             | 15.58               | 1.6         | 6.6                           | -    | -                   | 5.9  | -                              | -                           |
| 4-Hydroxy-3,5-dimethoxybenzaldehyde         | 16.61               | 1.8         | -                             | -    | -                   | 10.8 | 4.4                            | -                           |
| Unidentified compound                       | 16.80               | 1.0         | -                             | -    | -                   | -    | -                              | -                           |
| 1-( 4-Hydroxy-3,5-dimethoxyphenyl) ethanone | 17.43               | 23.6        | 26.1                          | 43.6 | 32.0                | 38.6 | 40.1                           | 14.4                        |
| 4-Hydroxy-3,5-dimethoxybenzoic acid         | 18.30               | 1.7         | -                             | -    | -                   | -    | -                              | -                           |
| Diisobutyl phthalate                        | 18.68               | -           | -                             | -    | -                   | -    | 7.8                            | -                           |
| Dibutyl phthalate                           | 19.71               | 1.0         | -                             | -    | -                   | 5.4  | 4.7                            | -                           |

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## МОНОМЕРНИ ФЕНОЛНИ СЪЕДИНЕНИЯ ОТ ОТПАДЪЧНИ ХИДРОЛИЗНИ ЛИГНОЦЕЛУЗОЗНИ МАТЕРИАЛИ

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(Резюме)

Проведена е алкална обработка (4 и 6 часа) на отпадъчни хидролизни лигноцелулозни материали. Получени са следните мономерни фенолни съединения: 2-метоксифенол, 3-етилфенол, 4-етил-2-метоксифенол, 2,6-диметоксифенол, 4-хидрокси-3-метоксибензалдеhid, 4-хидрокси-3,5-диметоксибензалдеhid, 1-(4-хидрокси-3-метоксифенил) етанон, 1-(4-хидрокси-3,5-диметоксифенил) етанон, и др. За екстрахиране на мономерните продукти са използвани толуен и етилацетат.

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