

Chemical and fractional composition of bio-oil obtained from *Arthrospira platensis* by hydrothermal liquefaction

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Abstract. This work is devoted to the study of chemical and fractional composition of the bio-oil obtained from *Arthrospira platensis* by hydrothermal liquefaction in the temperature range of 240-330 °C. For bio-oil analysis standard elemental analysis, ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (FT ICR MS) and thermogravimetric analysis were used. It was shown that the yield of bio-oil was increased from 12.4% at 240 °C to 37.2% at 330 °C. With temperature increasing the spectrum of compounds found in bio-oil using FT ICR MS narrows and moves to the region of low-molecular compounds. It was found that among the components containing nitrogen and oxygen, compounds containing 1 and 2 nitrogen atoms, as well as ON and O₂N₃ classes, dominate in the bio-oil. With the increase in the temperature of the liquefaction process, classes of compounds containing several oxygen atoms ON, O₂N₃, O₃N₂, ON₂, transforms into the classes N and N₂. By thermogravimetric analysis it was shown that the gasoline fraction (with boiling temperatures up to 200 °C) in bio-oil samples obtained from *Arthrospira platensis* at 240-330 °C was in the range of 20-22%. The fraction of light-boiling components in bio-oil was slightly decreased when the temperature of hydrothermal process was increased.

1. Introduction

In recent decades, the studies devoted to biofuel production from cyanobacteria/microalgae were focused mainly on cultivation methods. In this context, the main tasks were first of all the increasing of productivity and lipid content in biomass by strains selection [1] and cultivation conditions optimization [2]. At the same time, not enough attention was paid to the problem of biomass to biofuels conversion. The rational solution of this problem is one of the key ways to improve the energy efficiency of the process of biofuel production. The traditional conversion method usually includes drying, solvent extraction of lipids and transesterification with the production of fatty acid methyl esters composing the biodiesel fuel. Obvious disadvantages of biodiesel production method are high energy costs and the use of dangerous organic solvents (such as methanol). Moreover, in case of biodiesel production only lipid part of biomass is processed, while great part of biomass including



proteins and carbohydrates is not involved in the production of liquid biofuel. At the same time, it is known that lipid-rich strains have relatively low biomass productivity [3].

The problem of biofuel production is connected primarily with high moisture content when leaving the cultivation stage (80-90 % by weight). For the processing of wet biomass into biofuels the hydrothermal process seems to be more favorable (in general, a "hydrothermal process" means any process that takes place in the presence of water or steam above 100 °C [4-6]). Hydrothermal liquefaction (HTL) with the production of crude bio-oil (biocrude oil) as end product is of most interest [6]. One of the main advantages of HTL is that feedstock pre-drying is unnecessary in this case. Biomass raw can be fed into HTL reactor in humid state, e.g. in the form of an aqueous suspension. Another advantage is that by HTL the resulting bio-oil is composed not only from lipids but also from carbohydrates and proteins that increases the overall yield of the product. Additional advantages of HTL are relatively low temperatures of the process and the possibility of organizing the one-step continuous process.

During HTL the biomass is thermally treated under humid conditions at temperatures up to 370 °C and pressures up to 25 MPa. Due to this treatment, the biomass components are passed through hydrolysis and pyrolysis reactions forming a number of liquid hydrocarbons, both soluble and insoluble in water, as well as gaseous and solid reaction products. HTL products are bio-oil, aqueous solution, solid residue and gas product. The target product of HTL is the so-called "crude bio-oil" (biocrude) - liquid hydrocarbons separated from the solid phase and an aqueous solution.

After HTL the bio-oil, aqueous solution and solid residue are extracted from the reactor usually together as common condensed product. Solid residue is often separated by filtration or sedimentation. To separate bio-oil from aqueous solution in laboratory conditions the organic solvents are used. The yield and properties of bio-oil obtained from microalgae by hydrothermal liquefaction depends on many parameters including the strain of microalgae, temperature and duration of treatment, solvent etc.

Arthrospira platensis is well-known culture that is used in the production of many commercial products including healthy food supplements, food for animals, cosmetics and pharmaceutical products. It is grown usually in an open manner in a large scale. *A. platensis* is classified as cyanobacteria by International Code of Bacterial Nomenclature (ICNB), although according to the International Code of Nomenclature for Algae, Fungi and Plants (Melbourne Codex 2012) it is classified as blue-green microalgae.

Cyanobacteria/microalgae *A. platensis* is one of the most promising sources of renewable biofuel. When it is used as biomass raw for biofuel production it is often considered as microalgae. Advantages of *A. platensis* are relatively simple and cheap technologies for its cultivation and harvesting and lability of biochemical composition, i.e. predictable response to changes in the external environment (illumination, temperature, limitation and starvation of the main biogenic elements) [7]. The productivity of *A. platensis* and its oil (lipids) content may be ten times higher than that of terrestrial biomass [8].

Hydrothermal liquefaction of *A. platensis* has been studied early [9-10]. In [9] by the comparison of two methods – hydrothermal liquefaction and pyrolysis, it was established that the hydrothermal liquefaction method is more suitable for the conversion of biomass to liquid biofuel. In [10] the optimal conditions of hydrothermal liquefaction were established: 350 °C, 1 hour holding time and 20% biomass concentration in the initial aqueous suspension. In another work [11], in the study of hydrothermal liquefaction of *S. platensis*, the optimal temperature was determined as 300 °C (holding time at a maximum temperature of 30 min), which provides a 30% yield of bio-oil. In [10-11], in addition to standard elemental analysis, FT-IR spectroscopy and gas chromatography-mass spectrometry were used.

In this work, a study of chemical composition of the bio-oil obtained from *Arthrospira platensis* by hydrothermal liquefaction is presented. The study was conducted in the temperature range of 240-330 °C, with the use of standard elemental analysis and ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (FT ICR MS). The latter method allows in one spectrum to detect tens of thousands of compounds (without fractionation) and determine the gross formulas of the

identified molecules. Recently, the FT ICR MS was used in [12], where the bio-oil produced by hydrothermal liquefaction of *Nannochloropsis salina* at a temperature of 350 °C was investigated. It was shown that a large number of heteroatomic nitrogen-containing compounds compose the bio-oil, and the most common are pyrroles, pyridines, pyrazines, imidazoles and their derivatives, which are the products of protein decomposition. To study the bio-oil obtained from the biomass of *S. platensis*, FT ICR MS was previously used in [13], however, the study depending on temperature was not carried out yet. For fractional composition analysis in present study the thermogravimetric method was used.

2. Experimental

2.1. Initial biomass

HTL experiments were carried out with cyanobacterium *Arthrospira platensis*. We used *Arthrospira platensis* rsemsu 1/02-P strain with straight trichomes formed due to natural morphological variability during prolonged cultivation for 20 years under laboratory conditions.

Biomass was obtained by semi-continuous cultivation in open pond with volume of 500 l, illumination of $55 \pm 5 \mu\text{E}/(\text{m}^2 \times \text{sec})$, steady-lighting conditions; $T=21 \text{ }^\circ\text{C}$. Pond is equipped by near-surface mixing. *A. platensis* was cultivated using Zarrouk's medium: $\text{NaHCO}_3 - 16,8 \text{ g/l}$, $\text{KNO}_3 - 3,0 \text{ g/l}$, $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O} - 0,66 \text{ g/l}$, $\text{K}_2\text{SO}_4 - 1,0 \text{ g/l}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O} - 0,2 \text{ g/l}$, $\text{NaCl} - 1,0 \text{ g/l}$, $\text{CaCl}_2 - 0,04 \text{ g/l}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O} - 0,018 \text{ g/l}$, $\text{EDTA} - 0,08 \text{ g/l}$, Zarrouk's trace metal solution - 1.0 ml/l, (per 1 l of distilled water). Molecular genetics identification and phylogenetic analysis with a high bootstrap support level based on the nucleotide sequence of the 16S rRNA and ITS gene showed that this culture has a 100% similarity with *A. platensis* strains from the NCBI strain bank, i.e. belongs to the *A. platensis* species.

The elemental composition of *A. platensis* was determined using a VARIO EL III Elementar Analysensysteme GmbH elemental analyzer. The content of ash in *A. platensis* was determined by pyrolysis at 800 °C and mass measurements using an analytical balance Sartorius Cubis MSA324S. The results of elemental analysis (wt.% daf basis) and ash content determination for *A. platensis* are presented in Table 1. Biochemical composition of *A. platensis* is presented in Table 2.

Table 1. Elemental composition (wt.% daf basis) and ash content of *A. platensis*. Oxygen content is determined by difference.

Sample	<i>A. platensis</i>
C	49.86
H	7.29
O	27.56
N	11.3
S	3.99
Ash	6,0

Table 2. Biochemical composition of *Arthrospira platensis*, wt.%.

Sample	Proteins	Lipids	Carbohydrates
<i>A. platensis</i>	60,7	12,1	7,1

2.2. Hydrothermal treatment

Hydrothermal treatment of *A. platensis* was carried out on a laboratory plant, the scheme of which is shown in Fig. 1. The reactor represents an autoclave with volume of 500 cm³, the maximum exploitation temperature and pressure for the reactor are 400 °C and 25 MPa correspondingly. Reactor heating is external ohmic. The heating process (heating rate, maximum temperature and exposure time) is controlled by a PC operator using an automated control system (supplied by National Instruments).

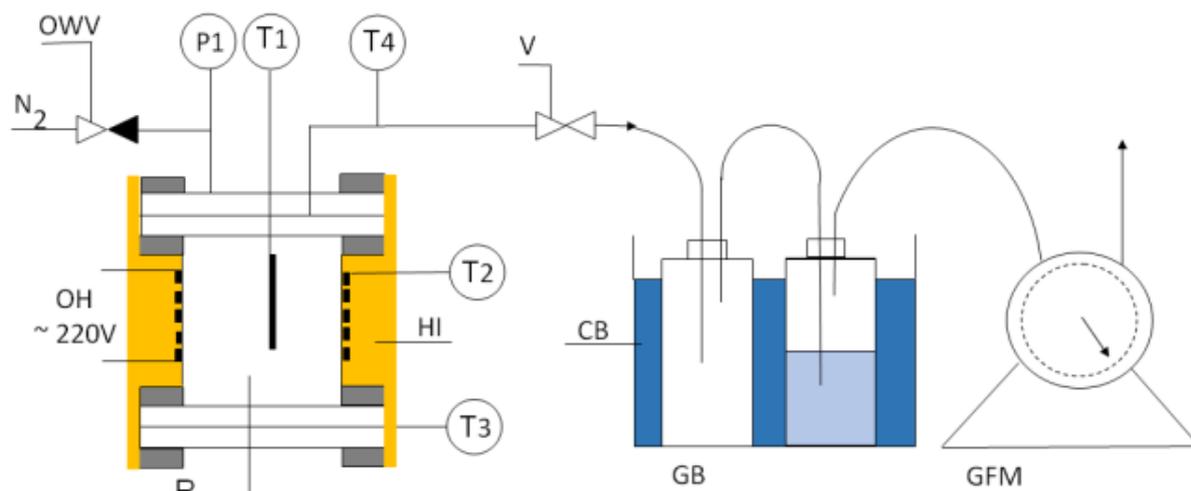


Figure 1. Scheme of a laboratory plant with reactor-autoclave. R – reactor, OH – ohmic heater, HI – heat insulation, CB – cooling bath, GB – gas bubblers, GFM – gas flow meter, OWV – one way valve, V – valve, T and P – temperature and pressure sensors.

Before hydrothermal treatment microalgae was centrifuged and dried at temperature of 80 °C. The reactor was loaded with 150 grams of distilled water and 30 grams of dried *A. platensis*. Then, the reactor was sealed and purged with nitrogen. Then, the reactor was heated up to a temperature of 240, 280 or 330 °C. The duration of the heating process to a maximum temperature was about 120 minutes, the exposure at maximum temperature was 60 minutes. In the reactor, a pressure close to the pressure of saturated water vapor corresponding to maximum temperature was established and maintained with the help of valve. In the end of exposure the ohmic heater was turned off and the reactor cooled to room temperature during about 5 hours.

Further, the reactor was opened and a solvent (dichloromethane) was poured into the reactor in an amount of 200 grams. After a day, about 100 ml of a solvent were removed from the bottom of the reactor with organic substances dissolved in it. The solvent was placed in an oven at 40 °C until the weight change of the residue ceased. The mass was measured using an analytical balance Sartorius Cubis MSA324S. The residue after evaporation of the solvent was a viscous liquid of a dark brown color. This product is referred to as bio-oil.

Bio-oil samples obtained at different temperatures process were studied on the Thermo Scientific Flash 2000 HT elemental analyzer and LTQFTUltra high-resolution mass spectrometer (ThermoElectronCorp., Bremen, Germany) equipped with a superconducting magnet (7 Tesla). The bio-oil was dissolved in the MeOH to the concentration 1g/l. Ions were generated by an IonMax Electrospray ion source (Thermo Electron Corp., Bremen, Germany) in positive and negative ESI mode. The temperature of the desolvating capillary was set to 300 °C. The infusion rate of the sample was 1 µl /min and the needle voltage was 3000 V. The archived resolving power was 400 000, each spectrum was the averaging of 100 scans. Prior to the analysis the LTQ FT was calibrated using the standard Thermo calibration mixture. The analysis of the obtained data was carried out using the previously described approach, which makes it possible to detect homologous series using the weighted mass defect histogram of Kendrick [13].

To assess the fractional composition, a thermogravimetric analysis of the samples of bio-oil obtained at different temperatures was carried out. The thermogravimetric analysis was carried out on a thermal analyzer STA PT1600 (Linseis Messgeraete GmbH). A sample of bio-oil in an amount of about 50 mg was placed in a corundum crucible. Heating of the crucible was carried out in argon. The heating rate to 500 °C was 2 °C/min, and then the heating rate to 800 °C was 5 °C/min.

3. Results and discussion

Table 3 shows the yield of bio-oil as a function of temperature. With temperature increasing, the yield of bio-oil increases: from 12.4% at 240 °C to 37.2% at 330 °C. Basing on the results of elemental analysis it can be supposed that at a temperature of 240 °C, mainly lipids contribute to the yield of bio-oil, whereas at temperatures of 280-330 °C, proteins and carbohydrates are subjected to hydrothermal liquefaction. The values of yield obtained in this work are in good agreement with the results of previous studies [10-11]. In [10], the yield of the light fraction of bio-oil at temperatures of 200 and 250 °C was about 18 and 25%, respectively. At the same time, the yield of the heavy fraction of bio-oil at these temperatures was insignificant. The maximum yield of bio-oil was reached at a temperature of 350 °C, at which the yields of light and heavy fractions amounted to about 14 and 25% respectively.

Table 3. The yield of bio-oil obtained from hydrothermal liquefaction of *A. platensis*, and its elemental composition (in the dry ash-free state), depending on the liquefaction temperature.

Parameter	Temperature, °C		
	240	280	330
Yield of bio-oil, %	12.4	26.5	37.2
C, %	65.5	69.1	73.7
H, %	6.1	6.0	6.0
O, %	22.3	18.0	11.1
N, %	3.5	4.1	2.8
S, %	2.6	2.8	3.0

Table 3 also shows the elemental composition of the bio-oil obtained from *A. platensis* by hydrothermal liquefaction. Compared with the initial biomass, higher carbon content and lower oxygen and nitrogen contents are observed in bio-oil samples. The hydrogen and sulfur content of the bio-oil is slightly lower than their concentration in the initial biomass. As the temperature increases, the carbon content increases. In bio-oil produced at 330 °C, the carbon content (for the dry ash-free state) is 73.7%. The oxygen content decreases with temperature increasing, but it remains still high (11.1% at 330 °C). The nitrogen content increases with temperature increasing, that agrees with the results of previous studies in this field [10].

Fig. 2 shows the mass spectra of the obtained samples of bio-oil. It is seen that if temperature increases, the spectrum becomes narrower and moves to the region of low-molecular compounds. With temperature increasing, the spectrum simplifies, the molecular-mass distribution takes the form of a Gaussian distribution, which resembles the mass spectrum of traditional oil [14].

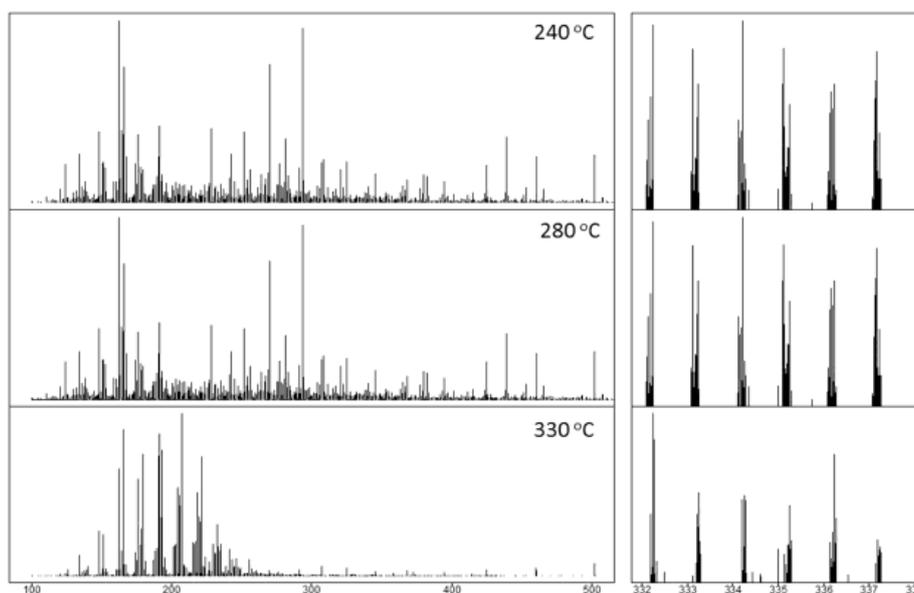


Figure 2. Mass-spectra of bio-oil samples obtained at different temperatures of the process of hydrothermal liquefaction of *Arthrospira platensis*.

For each of the samples, a Kendrick mass defect diagram, a weighted histogram of Kendrick mass defects were obtained, and the molecular formulas of the substances in the final products were determined. The results are shown in Fig. 3. The number of reliably determined molecular formulas after filtration and exclusion of isotopes was 1336, 1357 and 933 for temperatures of 240, 280 and 330 °C, respectively. It can be seen that compounds containing 1 and 2 nitrogen atoms dominate among all the nitrogen-containing and oxygen-containing components obtained in all the samples (at all temperatures), and also classes ON and O₂N₃ are present. It is seen that, at low process temperatures, the diversity of the classes of compounds obtained is much broader, intensive classes of compounds containing one or several oxygen atoms are observed: ON, O₂N₃, O₃N₂, ON₂ and others. With an increase in the process temperature to 330 °C, it can be observed that the intensity of these classes decreases significantly and only N and N₂ classes dominate in the spectrum, which, in general, agrees with the results of elemental analysis. It can be seen that the problem of a high nitrogen content in the bio-oil with a temperature change in the range 240-280 °C does not go away, it undergoes qualitative and quantitative changes, information on which can be useful in choosing the optimal method for further bio-oil processing.

Fig. 4 presents the results of the thermogravimetric analysis of the samples of bio-oil obtained at temperatures of 240 and 330 °C. It can be seen that the curves in the figure go close to each other. The curve for bio-oil obtained at 280 °C lies between the curves for 240 and 330 °C and isn't shown in fig. 4. The mass of samples after the heating to 800 °C decreases by approximately 80%. The decrease in the mass of the samples at temperatures of 300 °C and 400 °C is also almost the same and amounts to about 43 and 63% (for both shown curves), respectively. The main differences are in the temperature range up to 300 °C. The gasoline fraction (weight change to 200 °C) in the sample of biooil obtained at a lower temperature was about 22%, which is 2% higher than the sample of bio-oil obtained at a temperature of 330 °C. The fraction of light-boiling components (change in mass to 200 °C) is also higher in a sample of bio-oil obtained at a lower temperature. Thus, with the increase in the temperature of hydrothermal liquefaction, the contribution of the diesel fraction to the increase in the yield of bio-oil is greater than the contribution of the low-boiling fractions. This result agrees with the results of previous work [10], where it was shown that in the temperature range 250-350 °C the yield of the heavy fraction of bio-oil increases and the yield of the light fraction decreases.

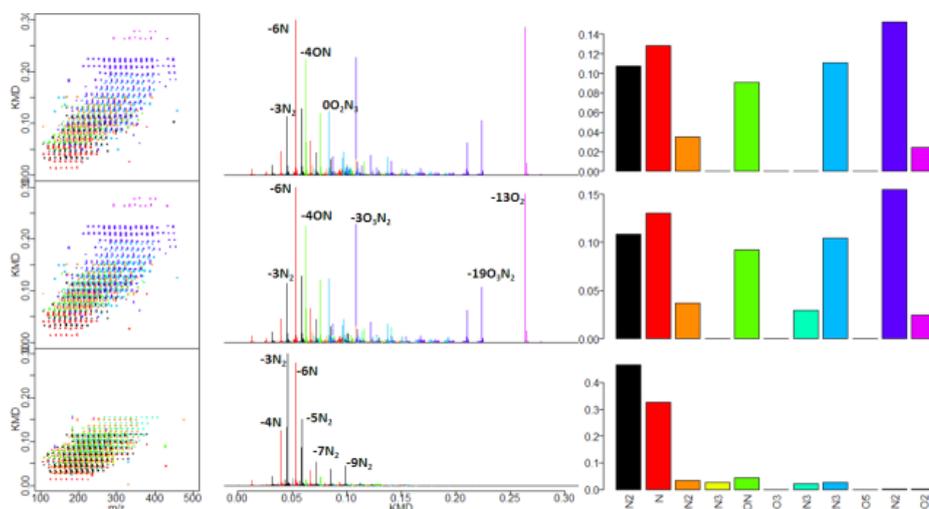


Figure 3. The visual analysis of the samples: Kendrick mass defect plot, weighted Kendrick mass defect histogram and portions of compound classes. Each class of compounds is labeled with unique color. The upper row of figures corresponds to the temperature of hydrothermal liquefaction of 240 °C, the intermediate row – to 280 °C, and the lower row – to 330 °C.

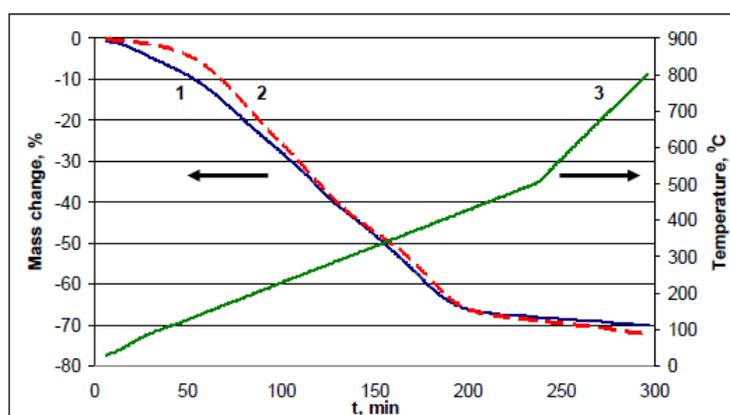


Figure 4. The change in the mass of the samples of bio-oil during the heating in argon medium: 1 - sample of bio-oil obtained at a temperature of 240 °C; 2 - sample of bio-oil obtained at a temperature of 330 °C; 3 - temperature versus time in the experiment.

4. Conclusion

With the change in the temperature of hydrothermal liquefaction of *Arthrospira platensis*, the yield increases and the chemical composition of the resulting bio-oil is also changed. An increase in temperature leads to an increase in the yield of bio-oil, a decrease in the oxygen content in it, and an increase in the content of carbon and nitrogen. The yield of bio-oil was increased from 12.4% at 240 °C to 37.2% at 330 °C. With temperature increasing the spectrum of compounds found in bio-oil using FT ICR MS narrows and moves to the region of low-molecular compounds. From the performed analysis of mass spectra, it was found that among the components containing nitrogen and oxygen, compounds containing 1 and 2 nitrogen atoms, as well as classes ON, O₂N₃, dominate in the bio-oil. With the increase in the temperature of the liquefaction process, classes of compounds containing several oxygen atoms ON, O₂N₃, O₃N₂, ON₂, transforms into the classes N and N₂. By thermogravimetric analysis it was shown that the gasoline fraction (with boiling temperatures up to 200 °C) in bio-oil samples obtained from *Arthrospira platensis* at 240-330 °C was in the range of 20-22%. The fraction of light-boiling components in bio-oil was slightly decreased when the temperature of hydrothermal process was increased. The fractions corresponded to boiling temperatures of 300 °C

and 400°C were almost the same for all bio-oil samples obtained (in the temperature range of 240-330°C) and equaled to about 43 and 63%, respectively.

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