Chemical Composition of Bio-oil Produced by Hydrothermal Liquefaction of Microalgae with Different Lipid Content

M S Vlaskin[1], Y I Kostyukevich[1, 2], G N Vladimirov[1, 2], M V Gaykovich[1*], A O Dudoladov[1], N I Chernova[1, 3], S V Kiseleva[1, 3], A Z Zhuk[1], O S Popel[1]and E N Nikolaev[2]

1 Joint Institute for High Temperatures of the Russian Academy of Sciences, Izhorskaya 13 Bldg 2, Moscow 125412, Russia
2 Skolkovo Institute of Science and Technology Novaya St., 100, Skolkovo 143025, Russia
3 Lomonosov Moscow State University, Leninskiye Gory, 1, Moscow 119991, Russia
E-mail: advert.geom@gmail.com

Abstract. Bio-oil samples were obtained by hydrothermal liquefaction at 300 ℃ of three different stains of microalgae that differ by the bimolecular composition: *Chlamydomonas*, *Chlamydopodium* and *Arthrospira*. For bio-oil characterization the ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (FT ICR MS) was used. The results of the study showed that the number of reliably identified molecular formulas after filtration and deisotoping were 2794 (*Chlamydomonas*), 1514 (*Chlamydopodium*) and 2110 (*Arthrospira*) and 853 formulas in each sample were the common. At the same time the compounds presented in the bio-oil produced from microalgae with high content of lipids and hydrocarbons (*Chlamydomonas* and *Chlamydopodium*) were more saturated then the compounds in bio-oil produced from *Arthrospira*. In the bio-oil produced from all microalgae the compounds containing 1 and 2 nitrogen atoms dominate, and also ON_2, N_3, ON_2 classes are present with relatively less intensity.

1. Introduction

Microalgae is one of the most promising sources of renewable biofuel [1]. The productivity of microalgae and its oil (lipids) content may be ten times higher than that of terrestrial biomass [2].
In recent decades, the studies devoted to biofuel production from 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 [3]-[5], cultivation conditions optimization [6]-[8] and new strains creation by genetic engineering. 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 [9]. 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 [10], [11].
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 technologies seems to be more favorable. Hydrothermal liquefaction (HTL) with the

production of crude bio-oil (biocrude oil) as end product is of most interest [12], [13]. 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 [14]. 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 ℃ 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.

In the present study we report the investigation of the bio-oil obtained by HTL of three different stains: *Chlamydomonas*, *Chlamydopodium* and *Arthrospira*. For bio-oil characterization the ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (FT ICR MS) was used.

2. Materials and methods

Bio-oil samples were obtained by HTL of three different stains of microalgae that differ by the bimolecular composition: *Chlamydomonas*, *Chlamydopodium* and *Arthrospira*. Bimolecular compositions of studied microalgae strains are shown in table 1.

Table 1. Biochemical composition of the investigated microalgae.

Strain	Proteins	Lipids	Hydrocarbons
Arthrospira platensis strain rsemsu 1/02-P	60.7	12.1	7.1
Chlamydopodium starrii strain rsemsu Chcc-14/11	29.1	34.9	31.4
Chlamydomonas globosa strain rsemsu Chlam-15/11	30.1	47.0	17.9

Hydrothermal treatment of microalgae was carried out on a laboratory plant, the scheme of which is shown in figure 1. The reactor represents an autoclave with volume of 500 cm^3, the maximum exploitation temperature and pressure for the reactor are 400 ℃ 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). Before hydrothermal treatment microalgae was centrifuged and dried at temperature of 80 ℃. The reactor was loaded with 150 grams of distilled water and 30 grams of dried microalgae. Then, the reactor was sealed and purged with nitrogen. Then, the reactor was heated up to a temperature of 300 ℃. The duration of the heating process to a temperature of 300 ℃ was about 120 minutes, the exposure at 300 ℃ was 60 minutes. In the reactor, a pressure close to the pressure of saturated water vapor corresponding to a temperature of 300 ℃ (closed to 100 bar) was established and maintained with the help of valve. Gaseous products of HTL were passed through gas bubblers where they cooled down and then were passed through gas flow meter. In the end of exposure the ohmic heater was turned off and the reactor cooled to room temperature during about 5 hours. The reactor was then opened and the condensed products (a mixture of an aqueous solution of organic compounds and a solid residue) were removed from the reactor and placed in a glass flask with flat bottom. Flask was placed on magnetic stirrer and the content within flask was stirred by magnetic element during 10 hours at room temperature to take additional organic compounds out from solid residue into aqueous phase. Then the stirring was stopped and the content within flask was left to form sediment on the bottom of the flask.

After sedimentation of solid residue on the bottom of the flask the aqueous solution was extracted from the flask using a syringe for further bio-oil extraction. For bio-oil extraction the dichloromethane was used.

Mass spectrometric analysis was carried out on a LTQ FT Ultra (Thermo Electron Corp., Bremen, Germany) mass-spectrometer equipped with a 7T superconducting magnet [15]. 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. For each peak in the peak list following variables were calculated:

$$\text{Kendrick mass } M_{Kendrick} = M_{IUPAC} \frac{\left[m_{IUPAC}^{CH_2}\right]}{m_{IUPAC}^{CH_2}} \quad (1)$$

$$\text{Kendrick mass defect } KMD = round(M_{Kendrick}) - M_{Kendrick} \quad (2)$$

Here [m] means integer part of the mass m. M_{IUPACK} is the IUPAC mass of peak, $m_{IUPAC}^{H_2}$ and $m_{IUPAC}^{CH_2}$ are IUPAC masses of the fragment CH_2 and H_2 correspondingly. Molecules that differ only by the number of repeating CH_2 segments have the same KMD. The homology series formed by C_cH_{2c} for molecules with molecular formula $C_cH_{2c+Z}N_nO_o$ are referred to as ZN_nO_o. Our approach for the spectrum processing is based on the analysis of the weighted Kendrick mass defect histogram. This diagram has the advantage over the conventional Kendrick mass defect plot (kmd vs. m/z) because it allow to see the homology series more distinct [16], [17].

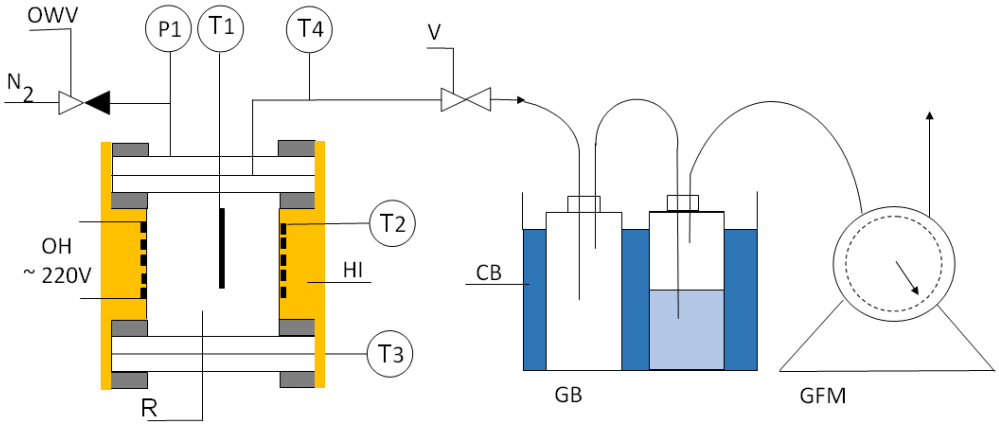

Figure 1. Scheme of a laboratory plant with reactor-autoclave. R – reactor, OH – ohmic heater, HI – heat insulation, CB – cooling bath, GB – gas bubblers, GFM – gas flow meter, OWV – one way valve, V – valve, T and P – temperature and pressure sensors.

3. Results and discussion

Results of the investigation of chemical composition of bio-oil produced from different microalgae are shown in figure 2. The number of reliably identified molecular formulas after filtration and deisotoping were 2794 (*Chlamydomonas*), 1514 (*Chlamydopodium*) and 2110 (*Arthrospira*). We can see that in the bio-oil produced from all microalgae the compounds containing 1 and 2 nitrogen atoms dominate, but also ON_2, N_3, ON_2 classes are present.

It can be seen, that the compounds presented in the bio-oil produced from microalgae with high content of lipids and hydrocarbons are more saturated then the compounds in bio-oil produced from *Arthrospira*. The number of double bonds in molecules spans the region from 1 to 7 for *Arthrospira* (maximum at 2) and the region from 1 to 10 for other microalgae (maximum at 7). Also, the portion of

oxygen containing compounds in *Chlamydopodium* and *Chlamydopodium* samples is considerably higher. Such results are expectable, because the *Arthrospira* has a considerable content of proteins (~60%) and as a consequence of nitrogen. The high content of nitrogen leads to the formation during HTL of the nitrogen containing compounds which easily ionize in the electrospray ion source.

Figure 3 presents the Venn diagram showing the intersection of the molecular formulas between bio-oil samples. It can be seen that the biggest number of unique molecular formulas (1157) is present in the bio-oil produced from *Chlamydomonas*. For all 3 samples 853 common formulas were found.

In the bio-oil produced from all microalgae the compounds containing 1 and 2 nitrogen atoms dominate, and also ON_2, N_3, ON_2 classes are present with relatively less intensity. Results show that the problem of high content of nitrogen remains for all bio-oil samples: the intensity of compounds containing 1 and 2 nitrogen atoms for bio-oil produced from microalgae with 30 % of proteins was almost the same as for bio-oil produced from microalgae with 60 % of proteins. Combustion of such bio-oil will lead to significant amount of NO_x to the atmosphere. It says that nitrogen reducing treatment of bio-oil is needed irrespectively of microalgae strain.

Our results suggest that the quality of the bio-oil obtained by the HTL depends on the composition of the biomass. High content of proteins leads to the obtaining of low saturated bio-oil with low content of oxygen.

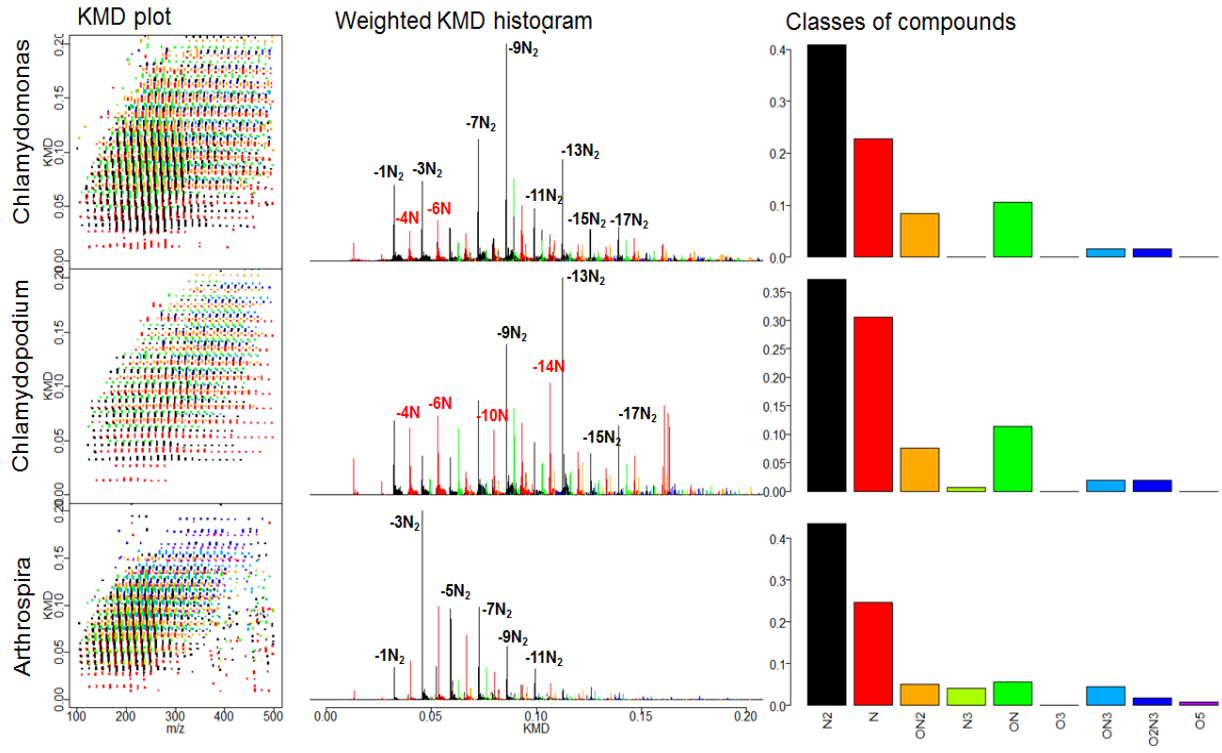

Figure. 2. The visual analysis of the samples: KMD plot, weighted KMD histogram and portions of compound classes. Each class of compounds is labeled with unique color.

4. Conclusion

Bio-oil samples were obtained by HTL of three different stains of microalgae that differ by the bimolecular composition: *Chlamydomonas*, *Chlamydopodium* and *Arthrospira*. The results of the

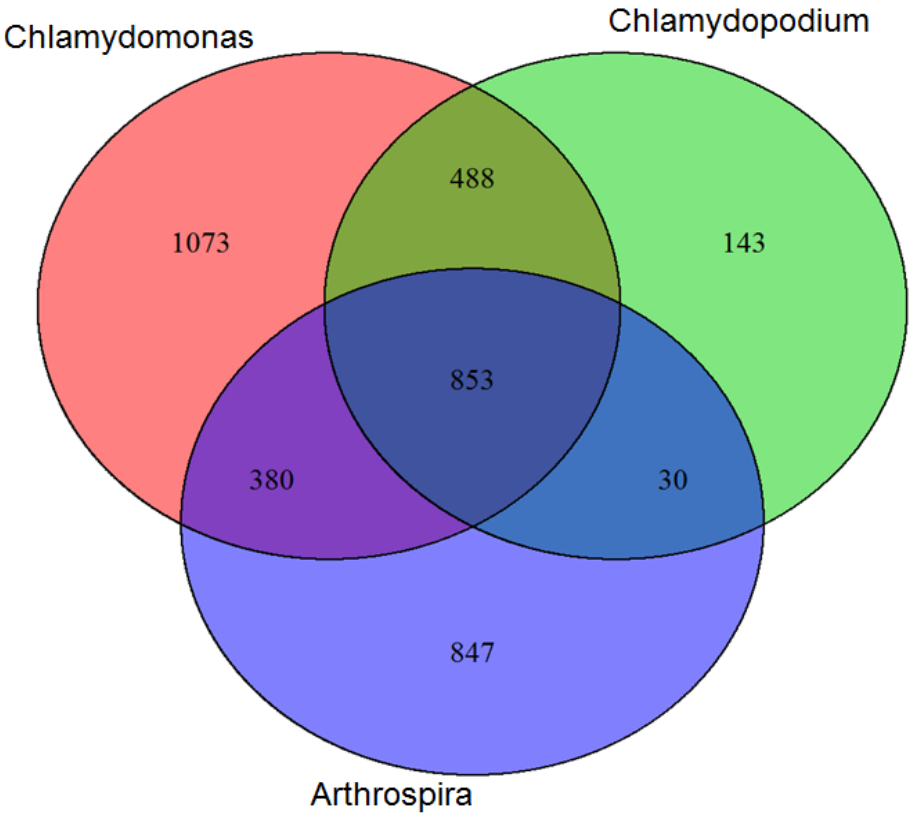

Figure 3. The Venn diagram showing intersection of compounds in different samples.

study showed that bio-oil samples produced from different microalgae differ from each other. The number of reliably identified molecular formulas after filtration and deisotoping were 2794 (Chlamydomonas), 1514 (*Chlamydopodium*) and 2110 (*Arthrospira*) and 853 formulas in each sample were the common.At the same time the compounds presented in the bio-oil produced from microalgae with high content of lipids and hydrocarbons (*Chlamydomonas* and *Chlamydopodium*) are more saturated then the compounds in bio-oil produced from *Arthrospira*. The obtained results can be used in the future for the processing of microalgae into bio-oil.

5. References

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