The wastewater using in technologies of bio-oil production from microalgae: CO₂ capture and storage

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Abstract. Bioenergy remains the largest renewable energy industry. At the same time, microalgae (MA) are a promising object of research among other types of biomass and the scale of microalgae using for energy purposes is increasing. Industrial cultivation of microalgae opens up great opportunities for CO₂ utilization and wastewater treatment from organic and mineral pollutants, and also significantly reduces the load on fresh water supplies. To reduce the cost of biofuels, optimization of a whole number of technological stages, including the cultivation of MA, is necessary. The paper presents the results of developing methods for cultivating MA to optimize their growth and absorption of nutrients from wastewater. A culture of microalgae/cyanobacteria Arthrospira platensis rsemsu P (collection of RSE Laboratory at Lomonosov MSU) which grew well in wastewater was experimentally selected. The results of the wet MA biomass conversion into bio-oil by hydrothermal liquefaction (HTL) technology with the associated production of biochar are presented. Biochar producing can be considered as a method for capturing and storing carbon.

1. Introduction
Nowadays the gradual movement of the energy sector towards the active development of renewable energy sources and the implementation of projects for carbon capture and storage (CCS) has become a global trend. In this regard, bioenergy can be considered as an effective solution of energy problems, as well as a promising approach to improving the environmental situation. Microalgae (MA) are considered a promising feedstock for the production of third and fourth generation biofuels, as they have higher growth rates than land biomass, and at the same time do not compete with food and feed production.

Due to the simple structure and simple type of nutrition, MA have a theoretically higher efficiency of photosynthesis (up to 12.6%) compared to terrestrial plants [1].

The lability of the MA biochemical composition allows obtaining biomass with desired properties and, accordingly, increasing the yield of biofuel of a given type (for example, the yield of biodiesel from biomass with an increased lipid content). Like other types of biomass, MA as feedstock for biofuel and energy production are “CO₂-neutral”.

Modern technologies for growing MA in open and closed systems can be optimized for the purpose of capturing and storing carbon in MA biomass - both short-term (biofuel production) and long-term (conversion of CO₂ into geologically stable forms of carbon). Approximately 1.83–1.88 kg of CO₂ can be fixed in 1 kg of microalgae biomass [2-3]. The industrial flue gas utilization for microalgae
cultivation has been already applied in breweries, cement factories and gas-fired power plants, as these factories generate gases with relatively low SO₂ and NO₂ concentrations [4]. The coal-derived flue gas has been also utilized for microalgae cultivation when it was diluted with air or added in small amount [4, 5-6]. A mitigation of industrial CO₂ emission from the unfiltered smoke stack of a 4MW coal-fired power plant through biotransformation into microalgal biomass with Desmodesmus sp. as the dominant microalgae was demonstrated in [7].

For the cultivation of microalgae, not only gaseous, but also liquid wastes (in particular, sewage) can be used, which makes it possible to reduce the need for water and nutrients by 90% [8]. Integration of biological wastewater treatment and CO₂ capture is a promising area of biofuel production from microalgae. The aim of this work is studying the effectiveness of wastewater treatment and CO₂ capture through the cultivation of microalgae and subsequent production of biofuel by the hydrothermal liquefaction of biomass.

2. Materials and methods

A microalgae Arthrospira platensis rsemsu P (Bios) with a high growth rate and the ability to respond to changing environmental conditions was selected for the study purposes. Arthrospira platensis is a 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. When it is used as biomass feedstock for biofuel production, it is often considered as microalgae [9-11]. A. platensis strain was obtained by semi-continuous cultivation in open pond with volume of 1000 l and illumination of 55 ± 5 μE/ (m² s) at steady-lighting conditions and temperature equal to T=21°C. The pond is equipped by near-surface mixing. A. platensis was cultivated using Zarrouk’s medium [12]. A. platensis strain has straight trichomes formed due to natural morphological variability during prolonged cultivation for 30 years under laboratory conditions. Microalgae are stably associated with heterotrophic bacteria, which we assigned to the genus Pseudomonas and Bacillus. These bacteria are able to assimilate dissolved organic matter and toxic elements and provide efficient municipal wastewater treatment.

The municipal waste waters of Moscow were used for experimental studies. The content of biogenic elements in the waste waters of Moscow, as well as typical and artificial waste waters (data from scientific publications) are presented in Table 2. It can be seen that the waste waters used in the experiments have a typical composition, but with an underestimated COD value, and an overestimated ammonium nitrogen content. The amount of nutrients (nitrogen ammonia, nitrates and orthophosphates) are present in a small amount (110, 2.4 and 15 mg/l, respectively) in comparison with the Zarrouk’s medium, which is optimal for the A. platensis cultivation. COD (total organic matter in heavily polluted effluents) 140 mgO/l and BOD₅ (readily oxidizable organic matter concentration in effluents) 85 mgO₂/l indicate that the sample contains a small amount of dissolved organic matter. According to the value of the hydrogen index (pH = 8.2), the wastewater is alkaline. The amount of oil products (2.7 mg/l) and total aromatic substances (0.668 mg/l) exceeds the average for this class of wastewater.

The A. platensis cultivation was carried out in undiluted and in diluted waste water (diluted by a factor of 2 ... 25) in Erlenmeyer flasks with a volume of 250–500 ml. The biomass growth rate of the same microalgae on the classic nutrient Zarrouk's medium served as a control [12]. The growing duration was 10–47 days. The degree of nutrients utilization from wastewater at different stages of the MA cultivation was controlled by chemical analyzes.

HTL experiments were carried out on the setup previously described at the [15] in collaboration with Joint Institute for High Temperatures of the Russian Academy of Sciences specialists. Hydrothermal liquefaction (HTL) with the production of bio-oil as end product is of most interest. 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 with water soluble organics, gas product and solid residue - biochar.

Table 1. The chemical composition of the municipal wastewater sample from the observation well of Moscow, artificial and typical waste waters

| Composition indicator, units | Waste water, Moscow | Artificial waste water [13] | Typical waste waters [14] |
|------------------------------|---------------------|-----------------------------|---------------------------|
| COD (bichromate oxidation), mg O/l | 140 | 749 | 900 | 820 | 798 | 925 | – | – |
| BOD₅, mgO₂/l | 85 | – | – | – | – | – | – | – |
| Potassium, mg/l | 15 | 2,1 | 8,9 | 3,9 | 11,7 | 17,4 | 4,5 | 5,4 |
| Nitrates, mg/l | 2,4 | 3,5 | 13,8 | 8,7 | 7,4 | 71,1 | 0,4 | 0,4 |
| Ammonium, mg/l | 110 | 26,9 | 53,6 | 85,2 | 12,0 | 14,9 | 42 | 56 |

3. Results and discussion

Due to the toxic compounds (phenols, ammonium, anionic surfactants) presence in the waste water, it was necessary to experimentally determine the degree of its dilution for sustainable growth of microalgae *A. platensis* with heterotrophic bacteria-associates. A steady growth of *A. platensis* was found for 12 days on undiluted municipal waste water but with the addition of 1/2 sodium bicarbonate doze from the composition of the Zarrouk's culture medium. Subsequently, the biomass accumulation lagged behind the control, and after 25 days the growth of arthospira cells in the wastewater stopped. A 5-fold dilution of waste water was determined as the highest, at which a consortium of *A. platensis* with associate bacteria can effectively grow and assimilate nutrients within 8 days. The sodium bicarbonate addition to the wastewater created more favorable conditions for the MA growth. Under dilution of wastewater by 3-1.5 times with the addition of sodium bicarbonate, the effective growth of this consortium of MA with associate bacteria was observed within 47 days. As a result, the concentration of nitrogen and phosphorus ions decreased to trace values. Moreover, the amount of readily soluble organic matter (BOD₅) decreased from 85 to 53 mgO₂/l, which indicates the ability of MA to switch to mixotrophic growth with the consumption of organic matter.

The wastewater treatment efficiency under microalgae cultivation was determined by analyzing the water chemical composition before and after growing microalgae (Table 2).

Table 2. The wastewater main chemical indicators before and after growing MA (growing time 47 days, dilution 3x-1,5x)

| Composition indicator, units | Experimental results | Relative error, % |
|------------------------------|----------------------|------------------|
| COD (bichromate oxidation), mgO/l | Initial concentration | Final concentration | 15 |
| BOD₅, mgO₂/l | 85 | 53 | 13 |
| Potassium, mg / l | 15 | 4 | 10 |
| Nitrates, mg / l | 2,4 | <0,1 | 20 |
| Ammonium, mg / l | 110 | 1,5 | 12 |

A comparative analysis of the wastewater main chemical indicators before and after growing MA showed that *A. platensis* is capable of efficient growth in slightly diluted wastewater. However, a prerequisite for growth is the creation of alkaline conditions in a nutrient medium by adding NaHCO₃ in an amount of at least 6 g/l. *A. platensis* cells completely consumed phosphorus from the wastewater in the form of orthophosphates and nitrogen in the form of ammonium and nitrates, the concentration of which decreased by 4, 73 and 24 times, respectively.
The grown microalgae biomass was subjected to hydrothermal liquefaction at temperatures of 270, 300 and 330°C. Hydrothermal liquefaction (HTL) allows to process wet biomass and to convert all carbon-containing components into fuel, the components of which are bio-oil, synthesis gas and coal (Table 3, Figure1).

Table 3. Conversion products of *A. platensis* biomass by hydrothermal liquefaction at different temperatures

| HTL-products | Temperature, °C | 270°C | 300°C | 330°C |
|--------------|----------------|-------|-------|-------|
| Bio-oil, %   |                | 34,6  | 38,8  | 45,7  |
| Syn-gas, %   |                | 12,7  | 14,8  | 17,5  |
| Biochar, %   |                | 28,7  | 27,4  | 26,0  |
| Aqua-phase, %|                | 24,0  | 19,0  | 10,8  |

As can be seen from Table 3, during the hydrothermal liquefaction of MA biochar is formed in a significant amount (from 26.0 to 28.7% depending on the process temperature). Consequently, the HTL technology provides not only the conversion of MA into biofuel, but also the removal of CO2 from the atmosphere by converting part of the carbon into a stable form that can be deposited for a long time [16]. Thus, to some extent, CO2 emissions from the cultivation and processing of MA are compensated. The aqueous phase formed after HTL as a by-product can also be partially utilized by microalgae as biogenic elements. Aqua-phase or aqueous solution is a by-product with low energy value and also needs to be processed. Previously we have described the recycling of nutrients from an aqueous solution after HTL [15].

![Figure 1. *A. platensis* conversion products by HTL-technology under various experimental conditions. Initial biomass of *A.platensis* (1), bio-oil (2) and biochar (3).](image)

4. Conclusion
Cultivation of MA using wastewater in combination with biofuel production is promising as the cost of nutrients (fertilizers), CO2 emissions and pressure on fresh water resources are reduced. Experimentally selected strains of microalgae (*Arthrospira platensis* rsemsu P (Bios)), capable of growing for a long time in municipal wastewater with efficient utilization of nutrients. By means of hydrothermal
liquefaction, the *Arthrospira* biomass, grown in wastewater, was transformed into biofuel (bio-oil and synthesis gas) and by-products – biochar and an aqueous phase. It has been proposed that biochar, which is 90% carbon and is a geologically stable form, should be considered as another alternative strategy for carbon capture and storage.

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