Phototrophic microorganisms biomass production with joint utilization of city surface water

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Abstract. Today surface water especially rain and thaw water became one of the environmental challenges for the cities. Those waters can be contaminated by different impurities including organics, oil products, heavy metals and etc. Utilization and reusing of those types surface water is the point of interest of many city agglomerations. In this paper preliminary results of Euglena gracilis CCAP 1224/5Z cultivation at highly organic contaminated surface water are presented. Cultivation was provided at illumination from 500 – 1200 lux depending on natural illumination in Moscow region. It was shown that during experiments that total organic carbon (TOC) content decreased from 41 mg/l to 15 mg/l in five days cultivation period. Final biomass concentration in case of cultivation on waste water exceeded concentration in the control flasks. Also the lipids content has been determined. In the biomass from flasks with surface water lipids content was from 24% to 32% to dry weight, depending on illumination and ambient temperature. Control biomass contains approximately from 28% to 30% in all experiments. Experiments with low TOC close to 10 mg/l showing close results – decreasing of TOC approximately at 50-60% in all flasks. Thereby it was demonstrated that it is possible in Moscow climate conditions during spring – autumn period to apply phototrophic microorganism E. gracilis strain for surface water treatment and in this case total lipids content a stay the same as in control cultivations.

1. Introduction

Population growth in urban areas is one of the most significant trends of the 21st century, affecting global economic development, energy consumption, use of natural resources and human well-being. Globally, 3.6 billion people live in urban areas. All of these city dwellers need fresh water, but surprisingly little is known about where large cities get water or the effects of this infrastructure on the global hydrological cycle [1]. The concentration of substances and energy within cities is an inevitable consequence of the dense settlement and the residents’ needs for food, clothing and fuel, which in turn greatly affects the distribution of nutrients on the planet [2]. Changes in chemical composition depend on various water sources. Urban runoff is considered to be the main reason responsible for the deterioration of water quality in the catchment areas [3]. Changes in the catchment hydrology associated with urban development also cause canal expansions and purification, as well as the introduction of more suspended and dissolved sediments, such as heavy metals, into water bodies [4].

Despite the fact that the commercial use of microalgae for both large-scale production of biomass, such crops as Chlorella and Dunaliella, and for wastewater treatment, has about 80 years [5] currently, technologies based on phototrophic microorganisms are only beginning to play a significant role both in the industry of various bioproducts (from fertilizers and biofuels to substances for cosmetics and...
pharmacy), and as self-sufficient systems for normalizing the state of the environment. At the same time, considerable attention is being paid to the introduction of photobioreactors in the urban economy [6, 7].

In this case, microalgae are very attractive objects, as they can grow not only in fresh and salt water, but also in the wastewater, using the necessary nutrients for growth, thereby purifying it [8].

There are several concepts of today city water management that can be basis for further integration of phototrophic microorganisms technology [9, 10]. In this regard, great attention is paid to the development of criteria for the compliance of wastewater that can be returned to circulation, for the cultivation of microalgae, as well as quality of biomass produced and possibility of further biomass processing with receiving of various products [11].

The paper considers the possibility of obtaining biomass of *Euglena* on various types of effluent (supernatant fluid wastewater, suspended solid free-wastewater and suspended solid and microorganisms-free wastewater). The presence of solid suspended particles had a greater negative impact on growth than the presence of microorganisms, therefore, for effective management of the wastewater treatment process, it is necessary to remove solid suspended particles first [12]. Sewage treatment using *Euglena* and obtaining potential raw materials for the production of biofuels: after 8 days of cultivation, the content of ammonium nitrogen decreased by 98%, total nitrogen by 93%, orthophosphate by 85%, 66% of total phosphate and 92% of total organic carbon [13].

In the case of employing of microalgae cultivation for surface water treatment has another advantage compared with other methods - it is an effective utilization of carbon dioxide, that can be a good point to the zero-carbon discharge city development [14].

So, based on mentioned above information it is possible to say that phototrophic microorganisms can became a good option for treatment of different types of city waste waters. In current work we presented results of highly contaminated by organics surface water treatment by using *E. gracilis* CCAP 1224 / 5Z strain with focus on middle-Russian climate conditions.

2. Materials and methods

2.1. Microalgae strain, pre-culture cultivation condition

The culture of *Euglena gracilis* CCAP 1224/5Z from the Culture Collection of Algae and Protozoa (Scotland) was used in the experiments. The microalgae strain were cultured in 250 ml Erlenmeyer flask with 100 ml modified HUT nutrient media [15] at 20 ± 1°C. Artificial illumination was provided using 20 W daylight normalized fluorescent lamps, whose emission spectrum was in the range of 400–700 nm and a light intensity of approximately 60 µmol PAR m\(^{-2}\) s\(^{-1}\) under 14:10 (light:dark) photoperiod on a laboratory shaker at 100 rpm.

The composition of HUT nutrient media was as follows mg/l: 20 KH\(_2\)PO\(_4\), 288.8 NH\(_4\)Cl, 25 MgSO\(_4\)-7H\(_2\)O, 400 sodium acetate, 40 Yeast extract and 1 ml of stock solution Pfennig microelements [16]. The composition of stock solution microelements was as follows mg/l: 5000 EDTA, 2000 FeSO\(_4\)-7H\(_2\)O, 100 ZnSO\(_4\)-7H\(_2\)O, 30 MnCl\(_2\), 300 H\(_3\)BO\(_3\), 200 CaCl\(_2\)-6H\(_2\)O, 10 CuCl\(_2\), 20 NiCl\(_2\)-2H\(_2\)O, 20 Na\(_2\)MoO\(_4\)-2H\(_2\)O.

2.2. Experiment conditions

Water obtaining from natural water source – “Isetskoe water storage reservoir” (Russia, Sverdlovsk city).

All experiments have been conducted in Erlenmeyer flasks (500 mL) with 300 ml under constant temperature 20 ± 1°C. Artificial illumination was provided using 20 W daylight normalized fluorescent lamps, whose emission spectrum was in the range of 400–700 nm and a light intensity of approximately approximately 60 µmol PAR m\(^{-2}\)s\(^{-2}\) under 14:10 (light:dark) photoperiod on a laboratory shaker at 100 rpm.

The composition of nutrient media for control was a modified BBM nutrient media [17] as follows g/l: NaNO\(_3\) – 1,25, MgSO\(_4\) – 1, KH\(_2\)PO\(_4\) – 1,25 and 1 ml of Pfennig stock solution of microelements.
2.3. Extraction of lipids from microalgae biomass and obtaining of fatty acid methyl esters (FAME)

The samples were centrifuged for 10 min at 4000 rpm (centrifuge Awel 20 mf) to sediment biomass. When biomass was freezing at -70°C and next lyophilization (Labconco free zone 2.5) of this biomass were provided.

Lipids from algae dry biomass were extracted with chloroform – methanol solution (2:1) using Bligh and Dyer method. [18] FAMEs were produced by direct methanolysis according to the method described previously [19].

2.4. GLC analysis of FAME

Qualitative analysis of FAMEs was performed with a Bruker 340 GC gas chromatograph equipped with a flame ionization detector and SelectTMBiodiesel for a FAME quartz capillary column (30 m × 0.32 mm × 0.25 μm) under the following linear temperature programming: 190°C (3 min), a temperature increase of 3°C/min, 230°C (5 min). The temperatures of the injection system and detector were 250°C, the linear velocity of the carrier gas (nitrogen) was 20 cm/sec, and the split ratio was 1 : 20. The volume of the injected sample was equal to 1 μL. Fatty acids were identified by means via comparison of the retention time of the analyzed sample with that of a standard sample (FAME mix Supelco–37, Supelco, United States). The content of the individual fatty acids was determined with an internal standard (C_{17:0}) [20].

2.5. Determination of total organic and organic dissolved carbon

Determination of total organic carbon (TOC) and dissolved organic carbon (DOC) carried out by using national standard PND F 14.1:2:3:4.279-14 “Method for the determination of organic carbon and total nitrogen in drinking, natural and waste waters by the method of high-temperature oxidation using carbon and nitrogen analyzers”.

2.6. Growth monitoring

Growth was monitored by measuring of dry biomass weight. Dry weight was measured according to the APHA method 8111G, [21] with a modified drying temperature (105 °C) and filter (Whatman GF/ C, 1.2 μm).

3. Results and Discussion

Algae growth was measured in terms of increase in biomass concentration (g L⁻¹) and is shown in Fig. 1. Initially two samples of surface water and control were inoculated with the same initial cellular concentration of E. gracilis. There was mild initial lag phase observed during the growth for 1 day of Euglena. Cultivation in the both cases of control and surface water, from the first day there is a gradual even growth. With the same starting density of 0.033 g L⁻¹, the dry biomass cell weight of the algae cells went up to 0.35 g L⁻¹ and 0.37 g L⁻¹ on the 5th day for cultivation on control medium and surface water, respectively.
Total organic carbon is one of the most important total parameters characterizing organic water pollution. This parameter has its exact definition and is an absolute value representing the content of all organic compounds. In this work surface water was used in which the main part (about 90%) DOC is constituted of TOC. During the experiments investigation photo-heterotrophic cultivation of *E. gracilis* was performed in order to reduce organic impurities and results shown in Fig 2. After cultivation of microalgae the contents of TOC and DOC is decrease for 63.4% and 64.8% respectively. In research high efficiency (about 90%) of reducing TOC for 6 days by cultivating *Euglena* in wastewater is shown [13].

As known, the amount and composition of lipids in a cell is affected by the state of the culture [22]. The total lipid content of the samples is presented in Fig. 3. In the biomass from flasks with surface water lipids content was from 24% to 32% to dry weight biomass, depending on illumination and ambient temperature. Control biomass contains approximately from 28% to 30% to dry weight biomass in all experiments. It should be noted the same result 27,2% of total lipid content is was noted earlier by the heterotrophic cultivation of *E. gracilis*, [23].
The quality of biofuels directly depends on the composition of fatty acids (FA), the main factors are the chain length and the degree of unsaturation. The results of determining the composition of fatty acids are presented in Table 1.

**Table 1.** Percentage composition of FAME: 1 – cultivation on control medium and 2 – cultivation on surface water.

| FAME composition | 1   | 2   |
|------------------|-----|-----|
| C₁₆:0            | 16,19 | 14,27 |
| C₁₇:0            | 18,06 | 7,34  |
| C₁₈:0            | 3,32  | 6,73  |
| C₁₈:1            | 6,06  | 5,40  |
| C₁₈:2            | 14,75 | 21,07 |
| C₁₈:3            | 38,49 | 33,14 |
| C₂₀:0            | 3,14  | 12,05 |
| Saturated FA     | 40,70 | 40,39 |
| Unsaturated, including: | 59,30 | 59,61 |
| Monoenoic FA     | 6,06  | 5,40  |
| Polyenoic FA     | 53,24 | 54,21 |

The composition of lipids was identified 8 fatty acids. C₁₈ FA: stearic acid (C₁₈:0), oleic acid (C₁₈:1), linoleic acid (C₁₈:2) and linolenic acid (C₁₈:3) prevailed in both the control and the experimental variants, with more than 50% being polyunsaturated fatty acids (PUFA): linoleic and linolenic. However, the relative content of these acids in the control and experimental variants differs. So, the C₁₈:3/C₁₈:2 ratio in the control was 2.61, and in the experimental variant - 1.57, which indicates a decrease in the activity of the desaturation processes in alga cells. Interesting is the fact that the content of saturated fatty acids in both variants is almost the same: 40.70% in the control and 40.39% in the test sample, while the content of margarine (C₁₇:0) acid in the experimental variant is almost 2.5 times lower than in the control, and the content of arachic acid (C₂₀:0) exceeded almost three times (12.05% and 3.14%, respectively), which may indicate more active elongation processes under the conditions of cultivation of the test sample.

European standards for biodiesel spelled out clear restrictions on the content of PUFAs, the share of which should not exceed 12% for linolenic acid, 1% with 4th and more double bonds. In our study, it was established that the content of linolenic acid in the amount of fatty acids of the lipids of the biomass...
of *E. gracilis* exceeded 30%. Similar results on the prevalence of linolenic acid in the cultivation of various microalgae in urban wastewater are presented in [24-26].

This high content of PUFAs causes a high instability of biofuels during storage and, undoubtedly, partial catalytic hydrogenation is necessary to increase its stability when used further as biodiesel [27].

4. Conclusions

- Demonstrated that it is possible in Moscow climate conditions during spring – autumn period to apply *E. gracilis* microalgae for surface water treatment and assess the TOC removal capability: after cultivation of microalgae the contents of TOC was decrease more than 60%;
- Maximum cell biomass after cultivation on a surface medium a bit more than in control medium;
- Evaluate the total lipid content and composition of FA profile of samples are determinates. For biomass samples growing on surface water and control medium content of lipids a stay the same and compose about 30%, but in the fa profile there are differences.

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