Possibilities of municipal waste water treatment by using phototrophic microorganisms under the Moscow climate conditions

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Possibilities of municipal waste water treatment by using phototrophic microorganisms under the Moscow climate conditions

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Abstract. Application of phototrophic microorganisms for municipal waste water treatment today became promising technology that combines solving of environmental issues and production of valuable biomass. This technology related to biomass cultivation in open ponds by using waste heat, that taken place in the waste water. Also huge volumes of municipal waste water makes economically impossible to apply artificial illumination. Thus application of phototrophic microorganisms for municipal waste water treatment is strongly related to climate conditions. In this paper data concerning phototrophic microorganisms cultivation in Moscow climate conditions are presented. Euglena gracilis CCAP 1224/5Z strain was used, cultivation was provided at illumination from 500 – 1200 lux depending on natural illumination. All cultivations were provided on synthetic waste water. It was shown that speed of growth was approximately the same in case synthetic waste water and controls. Content of organic impurities was decreased no less 70% at all rounds of cultivation. Final biomass concentration in case of cultivation on waste water exceeded concentration in the control flasks. Thereby it was demonstrated that it is possible in Moscow climate conditions during spring – autumn period to apply phototrophic microorganism E. gracilis CCAP 1224/5Z strain for waste water treatment.

1. Introduction
Lack of water resources and rising of waste water problems is one of the main challenges today. This is related with population growth, urbanization and climate change. Wastewater is contains a lot of organics and nutrients that must be removed before water is returned to natural water bodies to prevent eutrophication. Scientists are currently developing alternative treatment systems for wastewater treatment [1].

One of the most interesting directions of wastewater treatment is based on the microalgae cultivation. This type of system has several advantages over conventional chemical methods, such as high efficiency, lower costs, safety for the environment [2], and also because of their photosynthetic ability to convert solar energy into useful biomass and the use of nutrients such as nitrogen and phosphorus, causing eutrophication [3].

The types of nutrient sources that can be used for microalgae are municipal wastewater, breeding wastewater, industrial wastewater, and anaerobic digestion effluent. Nevertheless, the use of wastewater
as nutrients in buildings requires careful monitoring of the harmful chemicals potentially found in the wastewater, such as solvents, paints, and pharmaceuticals, in addition to dirt and mold [4].

The use of microalgae is effective not only for the removal of nitrogen and phosphorus from wastewater [5], but also there is possibility of obtaining biodiesel from biomass [2, 6], including application in remote areas [7]. It should be noted that different types of microalgae have a various ability to remove substances. Therefore, researchers suggest screening to determine the best strain for specific conditions [8, 9].

So in a number of scientific works there are examples of successful cultivation of microalgae in a particular city, belonging to different climatic zones. In the works, the possibility of growing *Chlorella* microalgae under conditions of a temperate climate belt passing to the sea, namely in the city of St. Petersburg, is shown [10]. Wastewater treatment and biomass production for biodiesel production at low temperatures (7–13°C), characteristic of the conditions of the North using cyanobacteria *Synechocystis* and *Chlorella* microalgae [11], cultivation of *Euglena* on wastewater in Tokyo conditions [12].

In the case of employing of microalgae cultivation for wastewater treatment has another advantage compared with other methods - it is an effective utilization of carbon dioxide, which is also very important for the cities [13].

This study, we examined the possibility of cultivating microalgae *Euglena gracilis* CCAP 1224/5Z strain in two types of wastewater, recycling organic and inorganic substances under the Moscow climate conditions.

2. Materials and methods

2.1. Microalgal 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 [14] at 22 ± 1°C under artificial illumination daylight fluorescent lamps (20 W, 6400K) light intensity 60 µmol PAR m² s⁻² under 14:10 (light and dark) photoperiod on a laboratory shaker at 100 rpm.

The composition of HUT nutrient media was as follows mg/l: 20 KH₂PO₄, 288.8 NH₄Cl, 25 MgSO₄·7H₂O, 400 sodium acetate, 40 Yeast extract and 1 ml of stock solution Pfennig microelements [15]. The composition of stock solution microelements was as follows mg/l: 5000 EDTA, 2000 FeSO₄·7H₂O, 100 ZnSO₄·7H₂O, 30 MnCl₂, 300 H₂BO₃, 200 CaCl₂·6H₂O, 10 CuCl₂, 20 NiCl₂·2H₂O, 20 Na₂MoO₄·2H₂O.

2.2. Experiment conditions

All experiments have been conducted in Erlenmeyer flasks (5000 mL) with 4000 ml of artificial waste water X1 and X1/2 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 60 µmol PAR m² s⁻² under 24 light photoperiod with air bubbling 300 ml/min. The composition of synthetic wastewater referred to as X1 was 1000 mg/L of glucose, 95.5 mg/L NH₄Cl, 56.3 mg/L urea, 22.6 mg/L KH₂PO₄, 12.6 mg/L, FeSO₄·7 H₂O, 309 mg/L NaHCO₃ and 35 mg/L yeast extract. The residual water called X1/2 has half the concentration of all chemical species of X1 [1] and the composition of control HUT nutrient media was describe above.

2.3. Methods for the determination of permanganate index

The samples were centrifuged for 10 min at 4000 rpm (centrifuge Awel 20 mf) to eliminate all of the microalgae. Supernatant was used for analysis.

Permanganate index was determined by using method, presented in the GOST R 55684-2013 “Drinking water. Methods for the determination of permanganate index”.
In a conical flask with a capacity of 250 cm$^3$ make 100 cm$^3$ of a thoroughly mixed sample of the analyzed water, a few pieces of porous porcelain, add 5 cm$^3$ of sulfuric acid solution, 10 cm$^3$ of a working solution of potassium permanganate. The contents of the flask are heated on an electric hotplate so that boiling comes no later than 5-7 minutes, and boil for 10 ± 2 minutes, closing with a small conical funnel to reduce evaporation. To the hot solution add 10 cm$^3$ of oxalic acid working solution. The bleached hot solution is titrated using a burette with a working solution of potassium permanganate until a pale pink color appears, lasting for about 30 s. Record the volume of the working solution of potassium permanganate consumed for titration ($V$, cm$^3$).

Permanganate index ($I_{\text{Mn}}$) in terms of atomic oxygen, mgO$_2$/dm$^3$, is calculated by the equation:

$$I_{\text{Mn}} = (V_3 - V_0) \cdot C \cdot K \cdot 5 \cdot M / V_4,$$

where

- $V_3$ - the volume of the working solution of potassium permanganate consumed for the titration of an aliquot of the sample of the analyzed water;
- $V_0$ - the volume of the working solution of potassium permanganate consumed for titration at idle experiment;
- $C$ - concentration of potassium permanganate working solution, 2 mmole/dm$^3$;
- $K$ - coefficient correction for the working solution of potassium permanganate;
- 5 - stoichiometric coefficient;
- $M$ - atomic mass of oxygen for conversion to atomic oxygen equal to 8 g O$_2$ / mole;
- $V_4$ - titration amount of water analyzed, cm$^3$.

2.4. Methods for the determination of orthophosphate concentration

The samples were centrifuged for 10 min at 4000 rpm (centrifuge Awel 20 nf) to eliminate all of the microalgae. Supernatant used for analysis.

Orthophosphate concentration was determined by PND F 14.1:2.248-07 “Method of performance measurements mass concentrations of orthophosphates, polyphosphates and general phosphorus drinking, natural and waste water photometric method”.

The method is based on the interaction of orthophosphates with ammonium molybdate in an acidic medium with the formation of molybdophosphoric acid, its reduction by ascorbic acid in the presence of antimony chloride, followed by photometric measurement of the stained the blue color of the reduced form of molybdophosphoric acid (molybdenum blue) at wavelength 880 - 890 nm.

9.0 cm$^3$ of sample (or, when the content of orthophosphates is over 2.0 mg/dm$^3$ PO$_4$, its smaller volume diluted to 9.0 cm$^3$) is poured into a test tube with a screwed stopper, add 0.5 cm$^3$ mixed molybdenum-acid reagent and leave not less than 2 minutes. Then add 0.5 cm$^3$ of a solution of ascorbic acid, close the tube with a screw cap and mix. After 15–20 minutes, the measurement of the optical density (concentration, mg/dm$^3$) of the analyzed sample relative to the blank sample at a wavelength of 880–890 nm (spectrophotometer Thermo scientific Genesys 10S UV-Vis) is carried out. Distilled water is used as an idle sample through the entire course of the analysis.

To determine the concentration of orthophosphates in the sample, a calibration characteristic was established. According to the results, a calibration graph of the dependence of optical density on the concentration of orthophosphate ions (mg/dm$^3$) was made.

The mass concentration of orthophosphates (mg/dm$^3$ PO$_4$) in the analyzed sample is found according to the calibration curve, taking into account the preliminary dilution of the sample according to the equation:

$$X_{\text{PO}_4} = C_{\text{gr}} \cdot 10 / V_s,$$

where

- $X_{\text{PO}_4}$ - mass concentration of orthophosphates in the analyzed sample, mg/dm$^3$ PO$_4$;
- $C_{\text{gr}}$ - mass concentration of phosphates, found on the calibration curve, mg/dm$^3$;
- 10 - total volume of solution in a test tube, cm$^3$;
- $V_s$ - volume of water sample analyzed for analysis, cm$^3$.

2.5. Growth monitoring
Growth was monitored by dry biomass weight. Dry weight was measured according to the APHA method 8111G, [16] 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/dm³) and is shown in figure 1.

Initially two sample of wastewater X1 and X1/2 and control were inoculated with the same initial cellular concentration of *E. gracilis*. From the first day the culture on X1 and X1/2 medium has shown more rapid grow than control medium. This is consistent with the data obtained in [5]: since the beginning of the cultivation of the mixed indigenous microalgae at the primary effluent with a higher content of COD showed the fastest growth. The growing of *E. gracilis* on a wastewater are higher when control to 10.8 and 27.4 percent for X1/2 and X1 respectively, which is directly related to the richer composition of nutrient media.

![Figure 1. Comparison of culture *E. gracilis* grow throughout different wastewater.](image1)

pH was measured for 12 days of culture medium with the purpose of understand its variations figure 2. Initial pH of HUT medium was 7.1 and for artificial wastewater were 8.8 and 9.16 for X1/2 and X1 culture medium respectively. For control medium pH values showed a consistent increase from pH 7.1 to 8.78, what gives accordance with to study [17] about *Euglena* as a source of lipids for potential use as biofuel and wastewater treatment. During cultivation on the X1/2 and X2 no significant change of pH was observed.

![Figure 2. Variations of pH of different types of wastewater.](image2)

During this investigation photo-heterotrophic cultivation of *E. gracilis* was performed in order to reduce organic impurities. COD removal efficiency was very similar for both sample of wastewater figure 3. Rapid decrease in COD was observed in first four days and more slowly uptake during next
days of cultivation. Significant COD removals were achieved in case of X1 and X1/2 wastewater and compose 73.7% and 72.4%, respectively.

![Figure 3. Variations of COD concentration during the experiment for different types of wastewaters.](image)

Phosphorus is one of the main pollutants pose a serious threat to the water quality and cause damage to aquatic life [18]. It is known that the microalgae possess an ability to eliminate metals, nitrates, phosphates. Figure 4 depicts the result for the removal efficiency of orthophosphate in the wastewater samples by microalgae. The concentration of orthophosphate in the wastewater samples X1, X1/2 and control were reduced from 25.1 mg/dm$^3$ to 7.6 mg/dm$^3$, or 69.7% of removal efficiency; from 15.5 mg/dm$^3$ to 5.7 mg/dm$^3$, or 63.2% of removal efficiency; from 23.0 mg/dm$^3$ to 10.6 mg/dm$^3$, or 53.9% of removal efficiency, respectively. In other study process of artificial wastewater treatment more effectively: the phosphate was reduced for 94% by *Chlorella vulgaris* cultivation [19].

![Figure 4. Variation of orthophosphate concentration during the experiment for different wastewaters.](image)

4. Conclusions
In current paper was presented next results:
- Our research demonstrated that *E. gracilis* can effectively growth in the two types of artificial wastewater.
- Microalgae exhibited great ability to eliminate nutrients: after cultivation the decrease COD were more 70% and orthophosphate more 60%.
- Maximum cell biomass after cultivation on a surface medium a bit more than control medium;
- Demonstrated that it is possible in Moscow climate conditions to apply *E. gracilis* microalgae for waste wastewater treatment.

Further research will be focused on optimization of cultivation processes, developing of photobioreactor prototype and determination of lipid content.
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