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The Technology of Pre-Purification Treatment of Municipal Wastewater Using Microalgae *Chlorella Vulgaris*

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The possibility of integrating systems for wastewater treatment and production of raw materials for renewable sources of energy (lipids) and biodegradable polymers (lactic acid) has been studied. Municipal wastewater from sewage facilities was used as nutrient medium for the cultivation of microalgae strains of *C. vulgaris IFR C-111* and *C. vulgaris Beijer IPPAS C-2*. Experimental research has shown that cultivation of *C. vulgaris*, strain *IFR C-111* generates a biomass of cells with intracellular lipid content of 14 % (mass.). Cultivation of a strain of *C. vulgaris Beijer IPPAS C-2* for 9 days allows to accumulate a biomass of cells with intracellular lipid content of 21% (mass.). Both strains succeeded in reducing the concentration of total nitrogen and phosphorus in wastewater by ≈50% over 4 days. It is established that the cultural liquid of microalgae can form the basis of the nutrient medium for the cultivation of bacteria *Lactobacillus casei B-3241*.

1. Introduction

Rapid development and population growth are some of the reasons for the tremendously increasing local loads on the environment. This makes wastewater treatment and efficiency of existing sewage plants one of the most important problems nowadays. Another promising direction of research is development of technologies of cultivation and processing of microalgae, which are considered a promising source of raw material for the production of renewable energy, biodegradable polymers, animal feed, etc. Various industries are implementing the idea of material flows integration with the aim of raising the level of energy and resource saving. However, to create optimal approaches to the use of natural resources and recycling of products of human activities a wide range of technical tasks need to be solved: development of new low-waste environmentally friendly technology and creation of mathematical models to simulate technical processes, to find their optimal regimes and control modes. Therefore, experimental studies that would provide additional information to address these tasks are extremely relevant.

Literature review, as presented in Table 1, shows that integration of systems for wastewater treatment and production of raw materials for renewable sources of energy not only solves the task of reducing environmental load, but also saves water and energy resources.

The object of this study was municipal wastewater, which was tested for suitability of its use as nutrient media for cultivation of microalgae *C. vulgaris*. Also, the possibility of using the resulting microalgae culture fluid for the production of lactic acid was evaluated.

Thus, the objective of this study was to determine the feasibility of municipal wastewater treatment using microalgae *C. vulgaris*.

The following tasks have been set and solved to achieve these objectives:

1. Experimental research into the dynamics of wastewater treatment;
2. Research into the feasibility of producing lactic acid from sewage water which has undergone preliminary microalgae treatment.

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Table 1: Literature review

Study	Strain	Type of sewage	Time, days	Changes in nitrogen content, %	Changes in phosphorus content, %	Lipid yield, %
Mata et al. (2014)	*Chlamydomonas sp.*	brewery's wastewater (20 %, v/v)& 700 mL of distilled water	24	-	-	54
Shin et al. (2014)	*Scenedesmus bijuga*	digested food wastewater municipal wastewater	20	83	91	35
Marjakangas et al. (2015)	*C. vulgaris CY5*	piggery wastewater	8	98	-	29
Zhan et al. (2016)	*C. vulgaris*	synchronous water	20	62	69	29
Tan et al. (2014)	*Chlorella pyrenoidosa*	digested starch processing wastewater	14	83	97	20
Casazza et al. (2016)	*C. vulgaris CCAP 211*	Winery wastewater (WWW) (60 %) & Bold Basal Medium	15	100	-	14
Wang et al. (2017)	*C. vulgaris*	sludge extracts, which was from the treatment of the synthetic wastewater containing 2,4,6-trichlorophenol	8	45	90	-
Zhao et al. (2016)	*Nanno chloropsis sp.*	urban sewage	13	96	94	-
Nugroho et al. (2014)	*C. vulgaris*	tofu industrial wastewater	10	84	59	-
Baglieri et al. (2016)	*C. vulgaris*	wastewater from the hydroponic cultivation of tomatoes	56	99 (nitrates) 83 (nitrogen ammonia)	94	-

2. Methods and materials

2.1 Wastewater characteristics

Samples of municipal wastewater (city with a population of ≈270.000 people), taken at urban wastewater treatment facilities with the capacity of ≈80.000 m^3/day, have been used: *sample No.1* - water received at the treatment facility (contains nitrogen (total nitrogen) TN≈61 mg/L and phosphorus (total phosphorus) TP≈38 mg/L); *sample No.2* - water received at the treatment facility after sedimentation (TN ≈32 mg/L and TP ≈11 mg/L); *sample No.3* - water after biological treatment (TN ≈0.3 mg/L and TP ≈7 mg/L).

2.2 Cultivation and concentration of *C. vulgaris* biomass

To assess the effectiveness of wastewater treatment (*sample No.1, sample No.2, sample No.3*) microalgae strains *C. vulgaris IFR C-111* and *C. vulgaris Beijer IPPAS C-2* were used. Microalgae were cultivated in the photobioreactor with a capacity of 2 L within 9 days at a temperature of 20-22 °C and average illuminance level of ≈14 kLx. Determination of nitrogen was carried out using Russian Federal Standard GOST 33045-2014 "Water. Methods for determination of nitrogen-containing substances". The amount of phosphorus was determined according to Federal Environmental Regulations 14.1;4.248-07 "Quantitative chemical analysis of waters. Technique for the measurement of mass concentrations of orthophosphates, polyphosphates and phosphorus". The concentration of cells was determined by direct count in the Goryaev chamber using a light microscope "Levenhuk C310 NG" (×1000-1600). The biomass was concentrated to a moisture content of 95÷98 % using a centrifuge with rotation speed 3000 min^{-1} for 5 minutes.

2.3 Microalgae cell disruption and lipid extraction

Cell disruption was carried out by treating 100 mL of microalgae biomass with 98 % moisture content with a mix of enzymes "Cellolux A" and "Protosubtilin G3x", taken in the ratio 75 %:25 % (0.012 mg/mL – 0.004 mg/mL) for 10 minutes at a temperature of ≈50 °C, and then exposing it to microwave radiation of 280 W power for 40 s.
Extraction of lipids from microalgae cells was carried out similarly to the Bligh and Dyer method, but with ethanol and petroleum ether in the ratio 2:1 (vol.) over 24 hours at a temperature of 22 °C, with a ratio of dry matter biomass (g) to the amount of solvent mix (mL) R=1:80. The solvent was distilled using a rotary evaporator IR-1 M3 at a temperature of distillation 85 °C and the speed of rotation of the flask 65 min^{-1}.
Estimation of lipids extracted from biomass was done by Zoellner and Kirsch method of determination of total lipids (Zoellner and Kirsch, 1962).

2.4 Cultivation of *Lactobacillus casei B-3241* biomass

To obtain the lactic acid the strain *of Lactobacillus casei B-3241* has been used; it was cultivated in 0.5 L containers in the culture fluid obtained after the cultivation of the microalgae *C. vulgaris* in wastewater (*sample No.1, sample No.2, sample No.3*). Beet molasses served as a source of reducing agents. The initial content of reducing agents in the solution was 5 % (vol.), cultivation temperature of 37 °C, initial pH=7. The cultivation was performed over the period of 5 days.

3. Results and discussion

Analysis of chemical composition of municipal wastewater (Figure 1) suggests the feasibility of their use as a nutrient medium (*sample No.*1, *sample No.*2, *sample No.*3) for the production of microalgae biomass and subsequent processing into technical lipids. The separated cell culture fluid contains ammonium salts, amino acids, B vitamins, cobalt, copper, manganese, molybdenum, iron, zinc, iodine and other trace elements. These components have a stimulating effect on lactic-acid bacteria, therefore, a study was conducted on the implementation of this culture fluid for the production of lactic acid using *Lactobacillus casei B-3241*.
The composition of wastewater and cell culture fluid (contents of nitrogen, phosphorus and heavy metals) will vary depending on the season due to different efficiency of the microorganisms, so the flows can either be further treated using activated sludge (in aeration tanks) or directly discharged into the river.

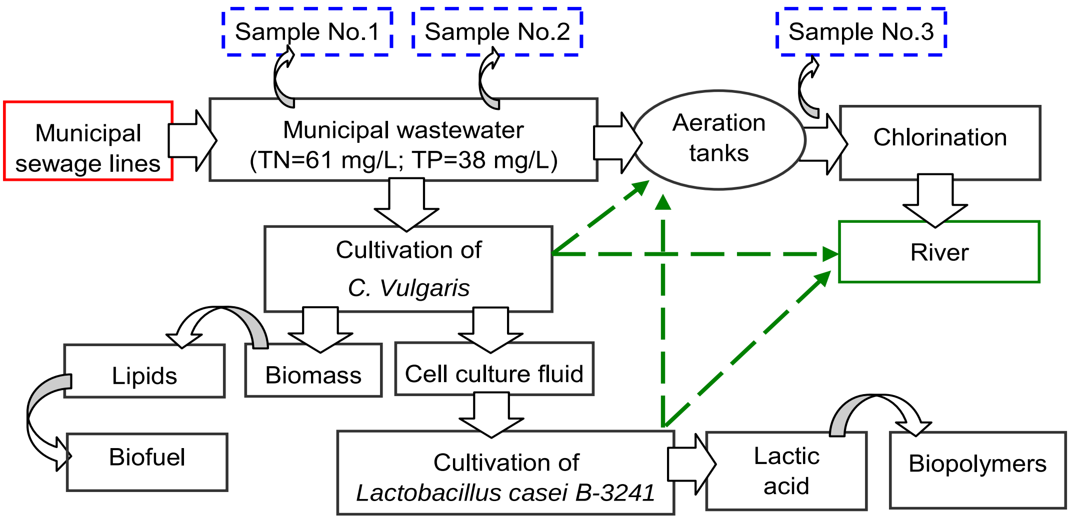

Figure 1: Flowchart of complex treatment of municipal wastewater

Analysis of experimental data on the cultivation of strains of microalgae *C. vulgaris IFR C-111* and *C. vulgaris Beijer IPPAS C-2* (Figures 2, 3) showed that the maximum biomass growth in the wastewater (1 - *sample No.*1, 2 - *sample No.*2, 3 - *sample No.*3) was observed during the cultivation *C. vulgaris Beijer IPPAS C-2* on the ninth day of cultivation (≈23 million cells/mL) and *C. vulgaris IFR C-111* on the fourth day (≈10 million cells/mL). Thus, to reduce the concentration of nitrogen and phosphorus to the level of TN≈30 mg/L and TP≈20 mg/L (Figures 4-7) it is advisable to use the strain *C. vulgaris IFR C-111*, as it accumulates biomass and lowers concentrations of TN and TP faster. The termination of the cultivation process on the fourth day is also advisable because the concentration of biogenic elements in the resulting culture fluid is sufficient for the cultivation of a strain of lactic-acid bacteria *Lactobacillus casei B-3241*.

Experiment on lipid extraction from wastewater-grown microalgae using integrated cell disruption methods (Dvoretsky et al., 2016) has allowed to conclude that the amount of lipids (Table 2) which can be extracted from biomass of *C. vulgaris Beijer IPPAS C-2* grown in wastewater (*sample No.*1, 2) is ≈21 % (mass.), and ≈14 % (mass.) for *C. vulgaris IFR C-111* (*sample No.*1, 2).

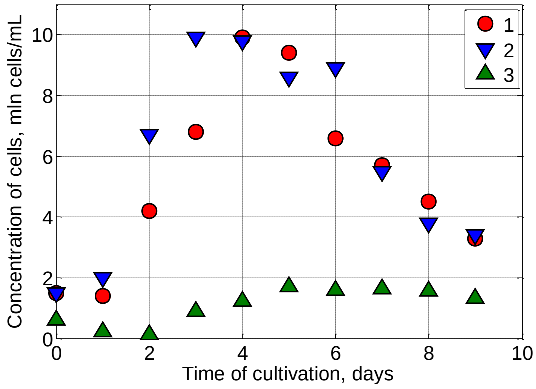

Figure 2: Dynamics of C. vulgaris IFR №C-111 biomass growth in different nutrient media

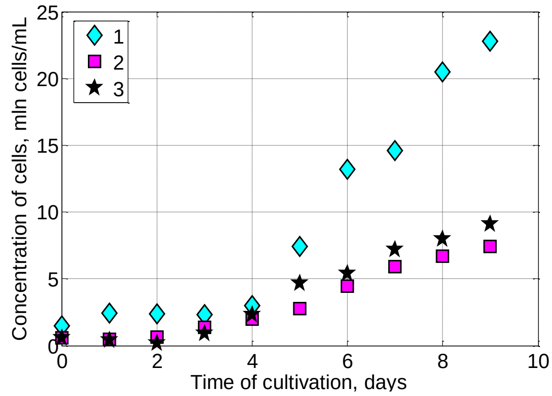

Figure 3: Dynamics of C. vulgaris Beijer IPPAS C-2 biomass growth in different nutrient media

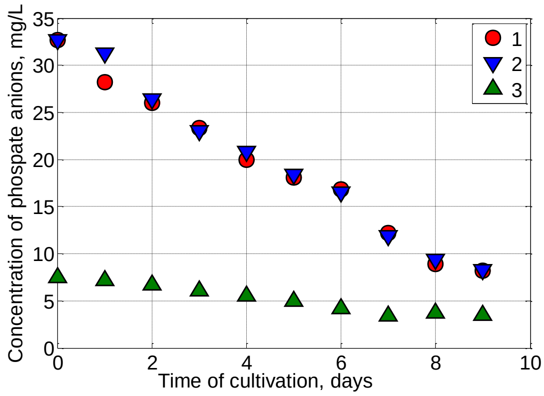

Figure 4: Kinetics of the changes in phosphate anions concentration in wastewater during the cultivation of C. vulgaris IFR C-111

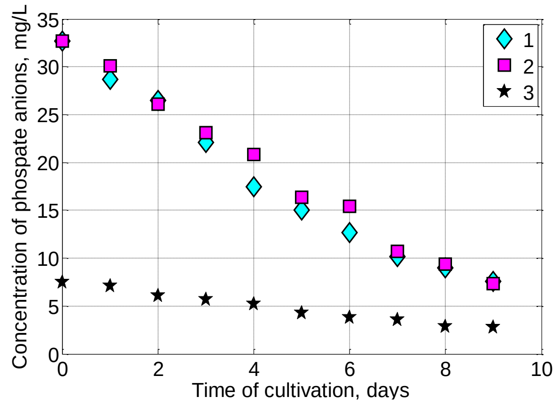

Figure 5: Kinetics of the changes in phosphate anions concentration in wastewater during the cultivation of C. vulgaris Beijer IPPAS C-2

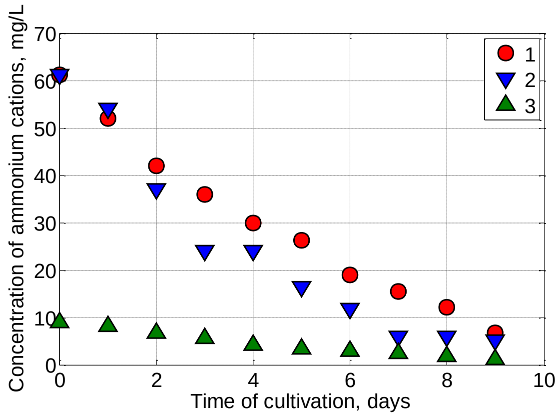

Figure 6: Kinetics of the changes in ammonium cations concentration in wastewater during the cultivation of C. vulgaris IFR C-111

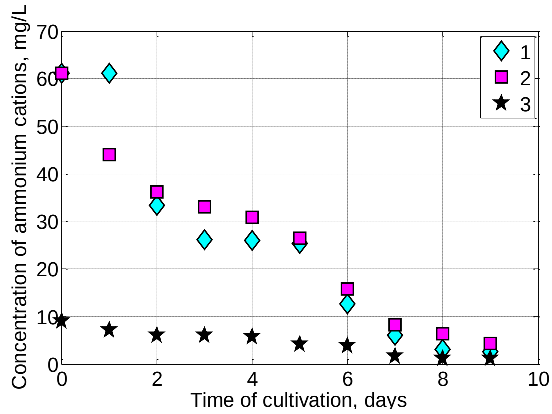

Figure 7: Kinetics of the changes in ammonium cations concentration in wastewater during the cultivation of C. vulgaris Beijer IPPAS C-2

The accumulation of biomass of *C. vulgaris IFR C-111* and *C. vulgaris Beijer IPPAS C-2* strains when cultivated in wastewater after biological treatment (*sample No.*3) amounted to an average of 2 million cells/mL for *C. vulgaris IFR C-111* and 9 million cells/mL for *C. vulgaris Beijer IPPAS C-2*. Smaller growth of cells can be explained by the fact that a smaller quantity of nutrients (nitrogen and phosphorus) is present in the wastewater after biological treatment. The yield of lipids during their extraction following the integrated cell disruption of *C. vulgaris Beijer IPPAS C-2* biomass was 24.0 %, and for *C. vulgaris IFR C-111* it amounted to ≈17.0 %. The increase in lipid yield can be explained by the fact that the cultivation of strains was carried out under stress conditions (nitrogen and phosphorus deficit).

Thus, the lipid productivity of *C. vulgaris, strain IFR C-111* is ≈25 mg/(L·day), and the productivity of *C. vulgaris Beijer IPPAS C-2* is ≈7.4 mg/(L·day). This allows to conclude that for wastewater treatment and technical lipids production, the cultivation of microalgae *C. vulgaris, strain IFR C-111* is more practicable as it is better adapted to adverse cultivation conditions. It should be noted that the cultivation of *C. vulgaris IFR C- 111* in wastewater leads to the reduction in the lipid yield by 39% compared to optimal conditions (Dvoretsky et al., 2015), however, it excludes the cost of water, nutrients and heating.

Table 2: Lipid yield from the microalgae C. vulgaris biomass

Strain	Lipid yield depending of the type of wastewater, % mass.		
	Sample No.1	*Sample No.2*	*Sample No.3*
C. vulgaris Beijer IPPAS C-2	21.0	21.1	24.0
C. vulgaris IFR C-111	14.0	14.0	17.0

After the separation of biomass, the treated effluent (1 - *sample No.1; 2 - sample No.2; 3 - sample No.3; 4 – sample No.4* (culture fluid after the cultivation in a Tamiya OPTIMUM nutrient medium)) and molasses were utilized for the cultivation of *Lactobacillus casei B-3241*.

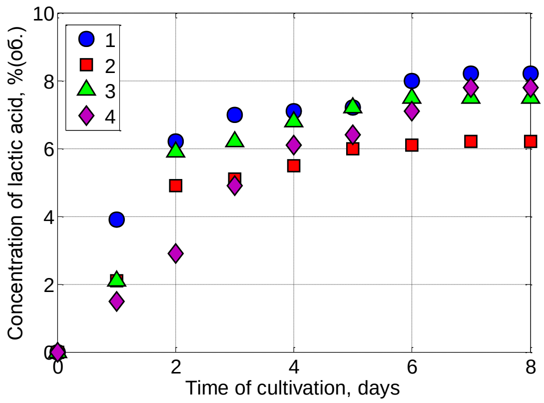

Figure 8: Kinetics of the accumulation of lactic acid in the cell culture fluid

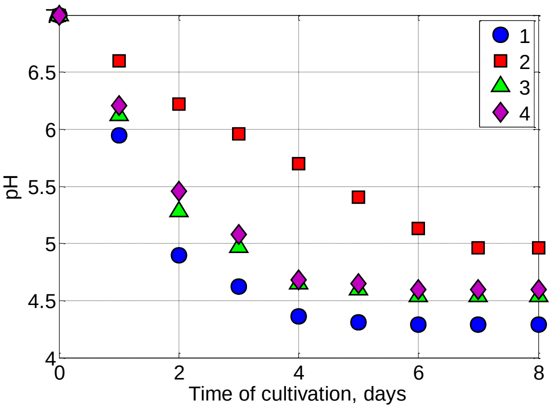

Figure 9: Kinetics of pH changes in the cell culture fluid

The maximum yield of lactic acid 8.2 % (vol.) was observed on seventh day (Figures 8 and 9) when using wastewater (*sample No.1*), which is 5% higher as compared with the yield of lactic acid during the cultivation of lactic-acid bacteria in the microalgae culture fluid - *sample No.4* . This can be explained by the fact that in this type of wastewater more ammonium nitrogen is left (graph 1 in Figure 6).

4. Conclusions

The results of the conducted experimental studies allow to conclude that municipal wastewater influent to the sewage facilities can be utilized, without pre-treatment and dilution, as a nutrient medium for the cultivation of microalgae *C. vulgaris IFR C-111* and *C. vulgaris Beijer IPPAS C-2*. The cultivated microalgae are considered a promising raw material for renewable sources of energy. The integration of wastewater treatment and their production saves water and other resources and reduces environmental load.

During the four days of cultivation of *C. vulgaris IFR C-111*, the biomass with cell concentration of 10 million cells/mL and intracellular lipid content 14 % (mass.) can be accumulated. The cultivation of the strain *C. vulgaris Beijer IPPAS C-2* for 9 days allows to accumulate a biomass of cells with a concentration of 23 million cells/mL and the content of intracellular lipids 21 % (mass.). Additionally, for both strains the concentration of total nitrogen and phosphorus decreases by 50 % in about four days. Culture fluid of microalgae can form the basis of the nutrient medium for the cultivation of *Lactobacillus casei B-3241*, and the maximum yield of lactic acid 8.2 % (vol.) has been observed on the seventh day.

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