

Experimental modeling of the microalgae cultivation in a photobioreactor using manure

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Abstract. The article studies the experimental process of cultivation of microalgae in a photobioreactor (PBR) to study the effect of technological conditions on the productivity of microalgae. This process allows obtaining initial data for the development of closed cycles of using the energy potential of alga mass in heat and power supply of various industries, including agricultural enterprises (livestock complexes, poultry farms, etc.) The scheme of a closed cycle of power supply of the cattle complex allows obtaining hot water, feed additives to the cattle ration, bio humus, motor biofuel and carbon dioxide, which is advisable to use in the process of cultivating microalgae. The experiments were carried out on a photobioreactor for cultivating microalgae with an intelligent control system. The developed photobioreactor differs from the known ones in the pulsating hydrodynamic regime of feeding the nutrient solution, which provides an increase in the productivity of the microalgae cultivation up to 15%. The experimental model of the cultivation conditions of the microalga *Ch. Vulgaris* on a combined diet (Tamiya medium + manure substrate) showed a noticeable increase in crop productivity when adding cattle manure extract to the nutrient medium in an amount from 30 to 60% (vol.). This can be used in the development of closed cycles of heat and power supply for cattle farms based on biofuels of the third generation, obtained from the phytomass of microalgae.

1 Introduction

The use of the peculiarities of the metabolism of microalgae, in particular, their high adaptability to changes in cultivation conditions, is an important independent task of the biofuel industry, since it opens up opportunities for the production of energy biological raw materials with specified physicochemical properties [1-5].

In addition, the industrial production of phytomass of microalgae for energy purposes using agricultural waste will effectively solve not only the problems of internal energy supply to farms, but also environmental problems in the agricultural sector. An example of such an integrated approach is an experimental study on the production of microalgae for energy purposes in the wastewater treatment cycle, conducted at the University of Virginia (USA) [6]. The scheme of a closed cycle of energy supply based on the phytomass of microalgae as an energy raw material obtained from the manure is shown in Fig. 1.

After washing and pressing in the preparation module, cattle manure is sent to a biogas plant to produce biogas and vermicomposting - a valuable fertilizer. Wastewater containing organic and mineral components is used as a nutrient medium for the cultivation of microalgae phytomass on a special farm,

which can be organized using open reservoirs or in glazed pavilions using heating and artificial lighting.

The produced phytomass is selected for processing, during which feed components (proteins, vitamins, trace elements) are extracted from it. The rest of the phytomass can be used for the preparation of composite boiler biofuels by adding petroleum products to waste in the process of homogenization using cavitation hydrodynamic and ultrasonic treatment. The resulting boiler biofuel differs from mineral boiler fuels in significantly higher environmental and performance indicators and can have a significant economic effect on the thermal economy of an agricultural enterprise.

If necessary, before being sent to the module for preparing composite boiler fuel, a lipid fraction can be isolated from phytomass, which, after purification, is added to mineral diesel fuel and, after appropriate processing in a homogenizing device, is used as a motor biofuel. The products of the technological cycle described above are biogas. It serves as a fuel for the generation of heat and electric energy, hot water and steam obtained in the boiler house when burning biofuel, feed additives to the cattle ration extracted from the phytomass of microalgae, biohumus. They can be used as fertilizers for growing feed, motor biofuel for the machine and tractor fleet of the enterprise and carbon dioxide, which is advisable to be utilized in the process of cultivating microalgae.

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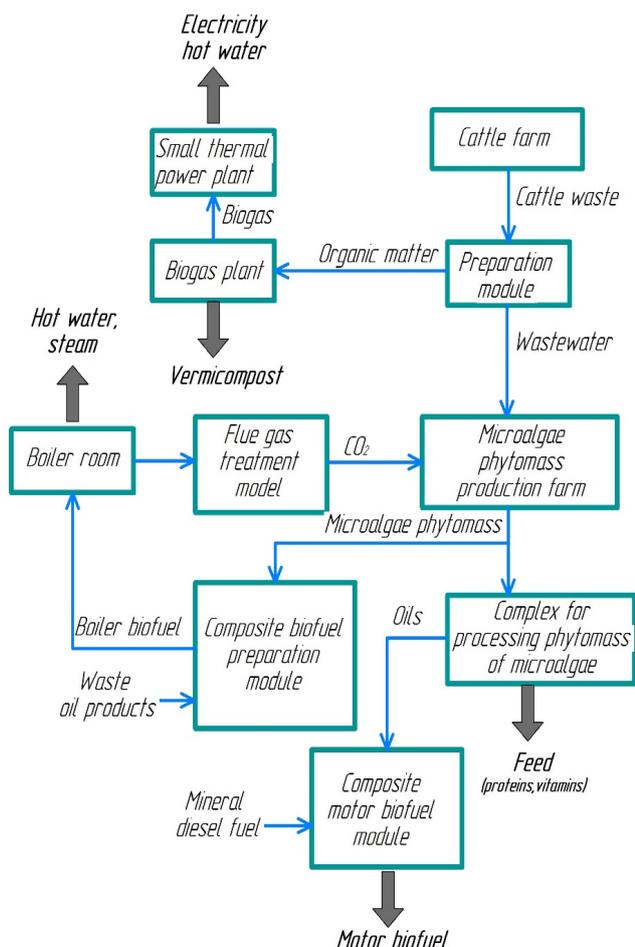


Fig. 1. Closed cycle of energy supply of the farm using phytomass microalgae as an energy raw material.

2 Materials and methods

To carry out experimental modeling of the cultivation processes, an inoculum of the microalga *Ch. Vulgaris* with a phytomass concentration of 7.5 g/L was used. The inoculum was diluted in a culture medium of two types. The first medium (*M*) contained only mineral substrate components, and the second (*MO*) was prepared on the basis of mineral and organic substances. As a mineral nutrient medium *M*, the standard composition of the Tamiya medium [7-8] is used. Mineral-organic environment *MO* was prepared from dry cattle manure without bedding. For this, the pounded manure substrate was placed in an ultrasonic capacity and poured with tap water in the ratio of 1 L water per 100 g of manure. The treatment in the bath was carried out for 10÷25 minutes, until a homogeneous suspension was obtained, after which the suspension was passed through a filter. The medium *MO* obtained in this way was stored in the cold place. Before use, the required amount of the medium was brought to a temperature of 18÷25°C. All experiments on the cultivation of microalgae were carried out in a 15.4 L photobioreactor in a quasi-continuous mode at the same average flow rate of the culture medium of 0.4 L/h. The flow rate of carbon dioxide was 0.2 g/min (0.1 L/min in terms of normal pressure). Microalgae were grown on nutrient mineral

and mineral-organic nutrient media at different ratios of substrates of the two compositions described above. Experiments were carried out on the cultivation of microalgae on nutrient medium without adding nutrient medium, as well as on their mixture in a volume ratio, respectively: 4:1, 3:1, 2:1, 1:1 and 1:2. The initial concentration of inoculum in all experiments was 0.45 g/L.

The initial concentration of inoculum in all experiments was 0.45 g/L. The density of the suspension in the absolutely dry mass of the ρ_{adm} at the bioreactor outlet was determined by the photometric method by the optical density at a wavelength of 0.75 μm . The bulk density of the suspension was determined empirically using a linear approximation of the optical density dependence [9]:

$$\rho_{adm} = (D_{750} + 0.08) / 1.42. \quad (1)$$

The green alga *Chlorella vulgaris* was chosen as the object of cultivation, which in the framework of this work is considered as the most acceptable type of phytoplankton for the industrial production of phytomass for energy use using a two-stage technology. A significant advantage of this (and derivatives from it) species is high competitiveness with respect to pathogenic microflora (blue-green) and high productivity, which is associated with the widespread occurrence in natural water bodies of the middle zone of the Russian Federation and high adaptability to biosystems typical of this climatic zone. *Chlorella* is one of the most studied phytoplankton species and is often used as a model object for studying the characteristics of the growth and reproduction of microorganisms [10-11]. As is known [12], cattle manure contains organic and mineral components that can be a source of biogenic components in the cultivation of microalgae. Since we are talking about the production of biofuel phytomass, there are no high requirements for sterility of production. The approximate composition of cattle manure is presented in Table 1.

Table 1. Composition of cattle manure.

Component	Content
Water	74,0
Organic matter	23,0
Total nitrogen	0,50
Ammonia nitrogen	0,15
Phosphorus (P ₂ O ₅)	0,20
Potassium (K ₂ O)	0,60

The process cultivation of microalgae was carried out in a continuous mode on an experimental setup that includes a cylindrical flow-through photobioreactor, a culture liquid recirculation system, a temperature control

system, a lighting system, a carbon dioxide saturation system and a ventilation system. The operation of all systems is carried out in an automatic mode and is regulated using an automatic control system. The photobioreactor for culturing the microalgae diagram is shown in Fig. 2.

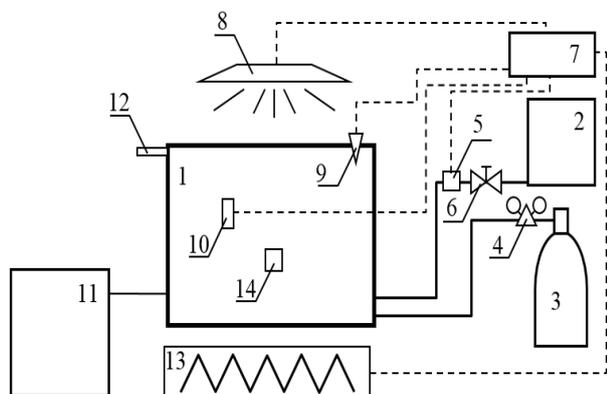


Fig. 2. The photobioreactor with an intellectualized process control system for the cultivation of microalgae: 1 - photobioreactor; 2 - raw material capacity; 3 – balloon with CO₂; 4 - gas reducer; 5 - flow sensor; 6 - fine adjustment valve; 7 - control system of cultivation parameters; 8 - lighting system; 9 - temperature sensor; 10 - microalgae concentration sensor; 11 - receiving container; 12 - branch pipe for the exit of excess gases (ventilation of the photobioreactor); 13 - thermostating system; 14 - dissolved CO₂ sensor.

The lighting subsystem consists of LED elements aimed at the tubes of the photobioreactor to ensure maximum light penetration onto the cultivation medium in the tubes. The LED illumination used in this work is assembled on LED elements of two colors located in the spectral regions required for plant cultivation (red and blue) [9].

The design of the photobioreactor shown in Fig. 2 is characterized by a relatively high value of the specific (per unit volume of the culture medium) light-receiving surface, which contributes to the efficient use of light energy. However, due to the final value of optical density, with a small diameter of the pipe, a certain part of this energy will pass through the reactor, without having time to be absorbed. For this, reflective screens are installed to organize the multiple reciprocating passage of light through the culture medium. In a combined lighting mode, in which, in addition to artificial light devices, solar radiation is used, the reflective screens illuminated by the sun are removable (Fig. 3).



Fig. 3. The photo lighting subsystem.

The feedstock supply subsystem consists of a feed tank, from which, through a fine adjustment valve, anaerobically processed organic waste necessary for the cultivation of microalgae, in a predetermined amount, enters the photobioreactor, as well as from the gas line, through which carbon dioxide is supplied from the cylinder through the gas reducer from the cylinder to the photobioreactor.

The thermostating subsystem has its own coolant and operates as follows. Inside some of the transparent tubes of the photobioreactor, in which the cultivation of microalgae takes place, stainless steel tubes are coaxially located, passing through the photobioreactor. The thermostated coolant circulates through these stainless tubes. The cultivation parameters control system is designed to control and adjust the cultivation parameters. The core of the system is an embedded system based on the microcontroller.

The system is equipped with the following set of sensors:

- 4 temperature sensors located evenly along the direction of the cultured liquid. If the temperature deviates from the set one, the temperature of the coolant of the thermostating system is adjusted (or the rate of circulation of the coolant increases);
- a flow sensor that measures the flow of anaerobically treated organic waste from the feed tank. If the speed deviates from the set one, an alarm occurs;
- a sensor for the concentration of microalgae, which serves to assess the growth of microalgae;
- dissolved CO₂ sensor.

The subsystem for controlling the cultivation parameters is also equipped with Bluetooth module for communication with a PC. The PC program was developed to visualize and record the parameters of the cultivation process in the National Instruments LabView environment. The developed program allows monitoring and recording in real time the readings of the feed solution flow sensor, dissolved CO₂ sensor, temperature and microalgae concentration sensors. It is also possible to change the set point of the heating medium temperature.

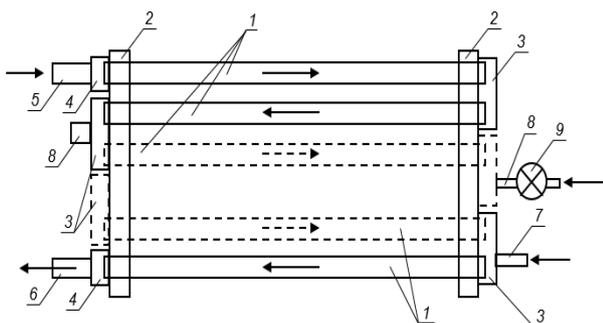


Fig. 4. Diagram of a tubular bioreactor operating in the displacement type: 1 - pipe; 2 - plate; 3 - jumper flange; 4 - flange; 5 - inlet fitting; 6 - outlet fitting; 7 - carbon dioxide supply connection; 8 - fitting for supplying mineral fertilizing; 9 - valve.

The photobioreactor for microalgae cultivation is equipped lighting, feeding raw materials, thermostating and monitoring cultivation parameters subsystems (Fig. 4). The photobioreactor differs from well known in the pulsating hydrodynamic regime of feeding the nutrient solution, which will ensure an increase in the productivity of microalgae cultivation up to 15%. To implement the process of cultivation of microalgae, it is necessary to maintain the following conditions:

- temperature of the cultivation process;
- CO₂ concentration in a suspension of microalgae in water;
- sufficient illumination of a suspension of microalgae in water with light, wavelengths of a certain spectrum.

3 Results and Discussion

Experiments were carried out to simulate the process of cultivation of the phytomass of microalgae. In these experiments, the operability of all systems of the photobioreactor was tested under typical growing conditions. The dependence of the productivity of microalgae on energy and substrate factors, in particular, the influence of the presence in the culture medium of mineral and organic substances in the form of extracts from cattle waste was studied. The cultivation conditions are shown in Table 2. The experimental results are shown in Fig. 5.

Table 2. Experimental cultivation conditions.

M	MO	T _{av} , °C	P, W/m ²	D ₇₅₀	ρ _{adm} , g/L
1	0	26,2	46	1,90	2,62
4	1	26,0		1,87	2,57
3	1	26,1		1,89	2,60
2	1	26,2		2,18	3,02
1	1	26,0		2,56	3,56
1	2	26,4		1,75	2,41

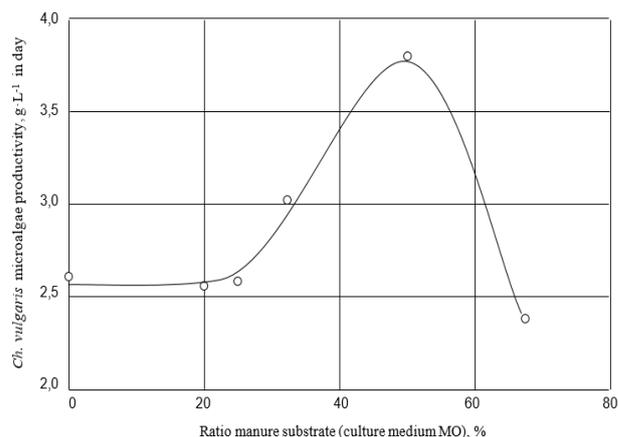


Fig. 5. The productivity curve of microalgae on the proportion of manure substrate in the nutrient medium

When conducting experiments on modeling the processes of cultivation of microalgae in a bioreactor, the influence of mineral and organic substances contained in livestock manure on the cultivation process and productivity of microalgae in artificial conditions was experimentally investigated. It is known that unicellular microorganisms are capable of not synthesizing biomass in the mixotrophs cycle when feeding on organic substances [9].

The study of the physico-biological mechanisms of microalgae mixotrophs can become the basis for the creation of methods for the purposeful correction of the chemical composition of the phytomass of biofuel microalgae and for reducing the energy intensity of the technological cycle of cultivation. As you can see from tables 5-3, the average temperature of the culture medium T_{av}, fluctuated in the series within a few tenths of a degree. This is due to the fact that, due to the change in air flows inside the room, it was not possible to more accurately thermostat the photobioreactor.

Nevertheless, these fluctuations are small and do not affect the reliability of the qualitative assessment of the simulation results. The dependence of the productivity of microalgae on the volume fraction of the manure extract in the culture medium is shown in fig. 3. The introduction of the extract into the culture medium in an amount up to 25% does not lead to noticeable changes in the productivity of the culture. Starting from a concentration of 30%, there is a significant increase in its productivity (about 30% of the average value), and with an increase in the proportion of manure extract to 65%, productivity decreases to a level below the value that was noted during cultivation on a completely mineral nutrient medium.

4 Conclusion

The experimental model of the cultivation conditions of the microalga *Ch. Vulgaris* based on a combined diet (*Tamiya* medium + manure substrate) revealed a noticeable increase in the productivity of the culture when the cattle manure extract was added to the nutrient medium in an amount of 30 to 60% (vol.). This is

possible due to the mixotrophs properties of the microbiological object under study and can be used in the development of closed cycles of heat and power supply for cattle farms based on third generation biofuels obtained from the phytomass of microalgae.

Acknowledgements

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