Energy intensity decrease in chlorella growth technology by electro ozonation

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Abstract. This paper examines process efficiency problems in chlorella microphytic alga growth for quality feed of livestock and poultry. Ozone-air mixture for microphytic alga growth simulation and development is being suggested for use. Paper describes experimental research methods realization and some experimental equipment. Experimental plant to carry out research was built, which produces ozone-air mixture, with required ozone concentration, and delivers it to the tank that contains chlorella microphytic alga suspension. Moreover, equipment was selected in a way so it doesn`t interfere with the main technological process of microphytic alga growth, special attention was given to an energy saving effect of the equipment and technology in general. Experimental research results discussion demonstrates some statistical data and dependence curves, constructed using these results, and also derived on this basis regression equation. Research results were processed by the "Statistika 6.0" software, obtained results indicate sufficiently close correlation relationship between examined data. Some experimental work results were summarized in the conclusions, and substantial efficiency of the suggested technology was verified, which allows to reduce energy consumption in forage production.

1. Introduction

Microphytic alga biomass addition as a vitamin feed supplement is one of many resource-saving technologies for animal feeding.

One of them is chlorella – a microscopic plant, that contains a large amount of protein, complete set of essential amino acids, carbohydrates, fats, vitamins and biological stimulants. Chlorella in agriculture is being used in a suspension form, which is prepared from the strain Chlorella vulgaris IGF S-111. However, in an agriculture in Russia, at present day, due to high energy intensity of a manufacturing process, usage of chlorella suspension is very limited. High energy intensity is explained by the use of a mercury arc lamps, to ensure photosynthesis. In order to produce 40-60 liters of suspension, this type of lamps consume from 12 to 24 kWh of electricity.

Therefore, chlorella manufacturers face necessity to increase productivity at a minimum production costs and eco-friendliness of a process. [1, 7, 10]

2. Materials and Methods

New experimental pant was built to carry out an experimental research. This equipment allows to produce ozone-air mixture with required ozone concentration and deliver it to the tank that contains chlorella microphytic alga suspension, which is being treated (Figure 1).

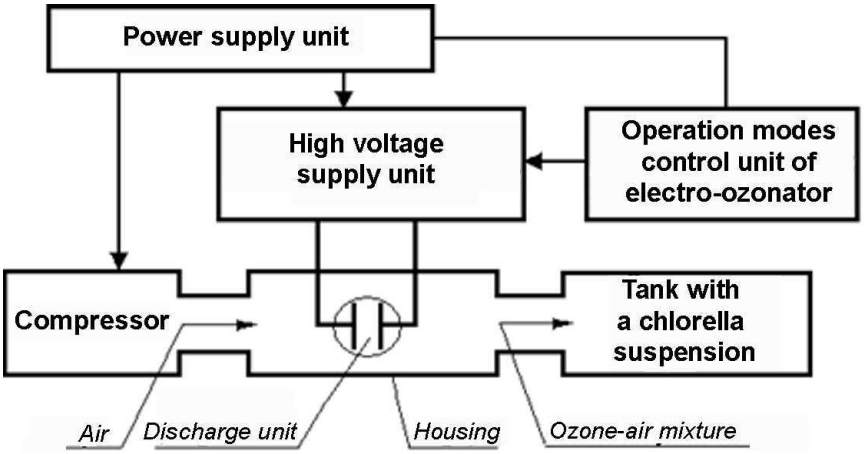

Figure 1. Functional diagram of the experimental plant.

Experimental plant consists of hermetically sealed housing of electro-ozonator, discharge unit, high voltage supply unit (high voltage transformer), laboratory autotransformer (LATR 1M), tank with chlorella suspension, flexible tubings, that connect all parts of the plant, and spray-diffusers.

Experiment was carried out at the FSBI HPE KubGAU laboratory, with the objective to detect influence of ozone-air mixture, at different values of concentration and exposure, on chlorella cells growth and development. First, searching experiment to determine location of ozone effective concentration area was conducted [2, 4, 12, 13]. During this experiment three tanks with chlorella suspension, 1000 ml each, were treated by ozone-air mixture, whose cells went through active fission and became «rejuvenated», with the following ozone concentration values 9.34 mg/m^3; 17.79 mg/ m^3; 40.04 mg/ m^3. Each tank was treated for 10 minutes. Tank number 4 was left untreated for control purpose. Experimental results tabulated in Table 1.

Table 1. Results of the experiment on ozone effective concentration area search.

Ozone concentration [O_3], mg/m^3	Treatment time τ, min	Number of cells under the camera's square N, pcs					Average number of cells under the camera's square Navg, pcs	Concentration of chlorella cells C_{chl}, c/ml
		1	2	3	4	5		
9.34	10	55	49	53	58	61	55	$13.75 \cdot 10^6$
17.79	10	43	55	34	39	57	46	$11.5 \cdot 10^6$
40.04	10	47	40	37	34	52	42	$10.5 \cdot 10^6$
-	-	59	48	63	36	56	52	$13.0 \cdot 10^6$

Analyzed test results suggest that in order to find an optimal area of ozone positive influence on chlorella cells it is necessary to examine carefully influence of the ozone-air mixture with ozone concentration with limits up to 12-13 mg/m^3, at a constant treatment time. [1, 8, 11]

To continue search for an optimal area of ozone positive influence, the following levels of ozone concentration were chosen for treatment: 3.2 mg/m^3; 5.2 mg/m^3; 7.2 mg/m^3; 9.2 mg/m^3; 11.2 mg/m^3; 13.2 mg/m^3. Experimental results presented in Table 2.

Table 2. Results of the experiment on ozone effective concentration area search.

Ozone concentration [O_3], mg/m^3	Treatment time τ, min	Number of cells under the camera’s square N, pcs					Average number of cells under the camera’s square Navg, pcs	Concentration of chlorella cells C_{chl}, c/ml
		1	2	3	4	5		
3.2	10	67	58	78	69	62	67	$16.75 \cdot 10^6$
5.2	10	82	72	64	73	65	71	$17.75 \cdot 10^6$
7.2	10	87	85	68	98	74	82	$20.5 \cdot 10^6$
9.2	10	77	76	74	72	62	72	$18.0 \cdot 10^6$
11.2	10	52	53	66	68	54	59	$14.75 \cdot 10^6$
13.2	10	51	55	54	75	48	57	$14.25 \cdot 10^6$
-	-	71	52	63	44	61	58	$14.5 \cdot 10^6$

Experimental results were used to construct dependence of chlorella cells concentration in suspension on concentration of ozone, which is being delivered into the tank. Conclusion was developed after the results analysis, that optimal area of ozone positive influence is within limits from 3.2 mg/m^3 to 9.2 mg/m^3 (Figure 2).

Determination of the effective treatment time by ozone on chlorella cells at concentration 7.2 mg/m^3 required the experiment with the following time levels: 2 min, 4 min, 6 min, 8 min, 10 min, 12 min, 14 min (Figure 3).

Using results of the experiment, also was constructed dependence of chlorella cells concentration in suspension on time of treatment by ozone-air mixture and presented pictures from Goryaev chamber of control and treated samples, made by digital camera Microscope Digital Camera Levenhuk C – Series for microscope, which were processed by Taup View software (Figure 4, 5).

As can be seen from the presented dependence, area of ozone effective influence on chlorella cells falls within limits of 4-10 minutes.

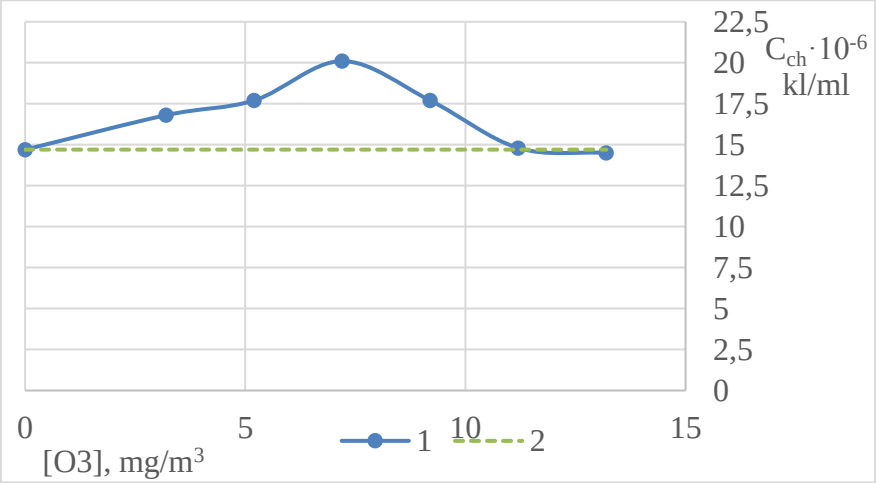

Figure 2. Data of the experiment on ozone effective concentration search, where 1 – cells treated by ozone-air mixture, 2 – untreated cells.

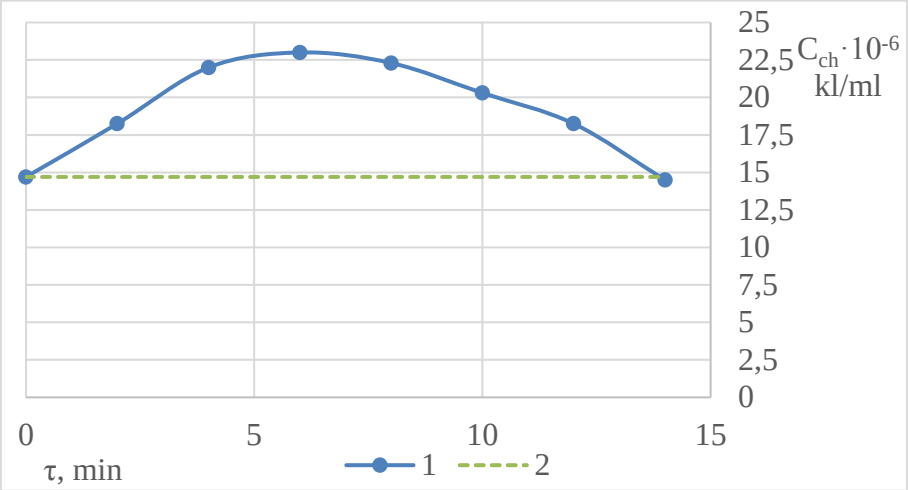

Figure 3. Dependence of chlorella cells concentration in suspension on time of treatment by ozone-air mixture, where 1 – cells treated by ozone-air mixture, 2 – untreated cells.

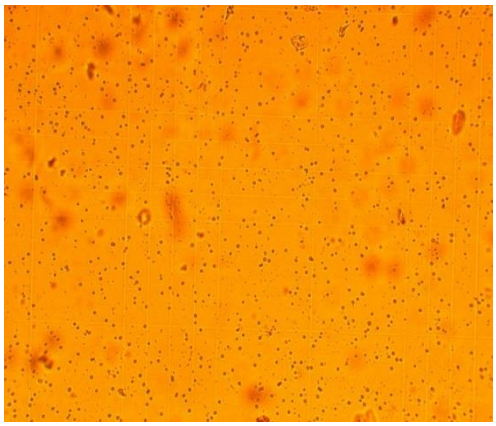

Figure 4. Control sample of chlorella suspension.

Figure5. Chlorella suspension after electro-ozone treatment with ozone concentration 7.2 mg/m^3 for 6 minutes.

Full factorial experiment was conducted, in order to determine ozone influence effect on chlorella microphytic alga cells more accurately [3, 5, 9, 14, 15]. This experiment was carried out with the help of mathematical statistic methods, the following is taken as an independent variable:

x_1 – τ – treatment time of chlorella suspension by ozone-air mixture (4 levels: 4; 6; 8; 10 minutes). Level values were taken in the extremum region, obtained from the single-factor searching experiment.

x_2 – [O_3] – ozone concentration in ozone-air mixture, which is being delivered into the tank with chlorella suspension (4 levels: 3.2; 5.2; 7.2; 9.2 mg/m^3). Level values were taken in the extremum region, obtained from the single-factor searching experiment.

x_3 – T – periodicity of chlorella cells suspension treatment by ozone-air mixture (4 levels: 1; 2; 3; 4 times in four days). Level values were taken with consideration of feed and fission cell bio cycles, and also specifics of chlorella microphytic alga cultivation.

During laboratory experiment as a dependent variable was taken y_1 – Cchl – chlorella cells concentration in one milliliter of undiluted suspension, c/ml.

Experiment planning matrix was compiled to carry out the experiment. Obtained from the experiment results were processed by mathematical statistic methods in «Statistika 6.0» software.

In order to determine an extent of influence of the electro-ozonation parameters on chlorella microphytic alga concentration the regression analysis was implemented, and as a result mathematical model was obtained in the form of multiple regression equation, which can be written as follows:

$$y_1 = 17,46 - 0,042x_1 + 0,48x_2 - 0,22x_3 + 0,38x_1x_2 - 0,55x_1x_3 + 0,3x_2x_3 - 0,086x_1x_2x_3 - 0,042x_1^2 + 0,48x_2^2 - 0,22x_3^2 - 0,042x_1^3 + 0,48x_2^3 - 0,22x_3^3 \quad (1)$$

As a result, was established close (R = 0.92) correlation relationship between cells concentration and factors being examined. Also was established that in 85% of the cases, factors, included in the equation, influence on the chlorella cells concentration, and in 15% - controlled by the other factors, which weren`t considered for a mathematical model construction.

Shift determines predicted value y_1, when all variables x equal 0 (in our case a = 17.46), and interpreted in the following way: typical cells concentration in one milliliter of untreated chlorella suspension solution is 17.46 million c/ml.

High significance level for the measure of cells concentration has the regression coefficient at x_2-variable (x_2 = 0.48, p = 0.0001). It shows that, all other things being equal, cells concentration is up by 0.48% due to treatment with certain ozone concentration. Also, substantial influence (0.3% and 0.38 respectively) on the cell concentration increase is done by the combination of factors, such as: $x_2 x_3$ (x_2x_3 = 0.3, p = 0.015) and $x_1 x_2$ ($x_2 x_3$ = 0.38, p = 0.07).

3. Results

Based on the results of the experiments, diagrams were constructed (Figure 6), which reflect influence of the factors being examined (treatment time x_1, ozone concentration x_2, number of treatments in four days x_3) on the independent variable y_1 (chlorella cells concentration in suspension).

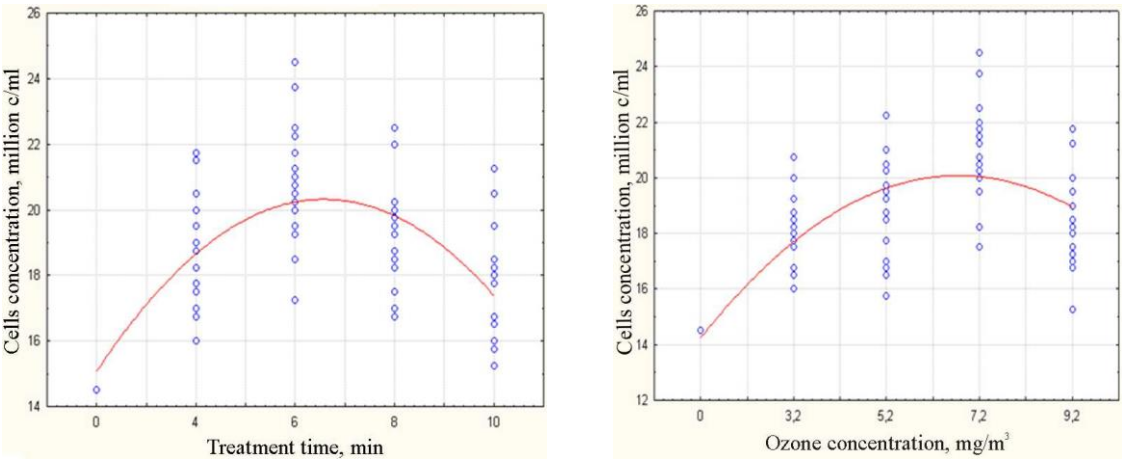

Figure 6. Diagram of influence of number of treatments in four days on the chlorella cells concentration in suspension

Analysis of Figure 6 leads to the conclusion that during treatment of chlorella cells suspension by ozone-air mixture, increase of chlorella cells concentration is observed up to ozone concentration of 7.2 mg/m^3 and treatment time 6 minutes. Further increase in ozone concentration and treatment time results in a smooth decrease in chlorella cells concentration, which is, probably, due to cells destruction from strong ozone oxidizing properties.

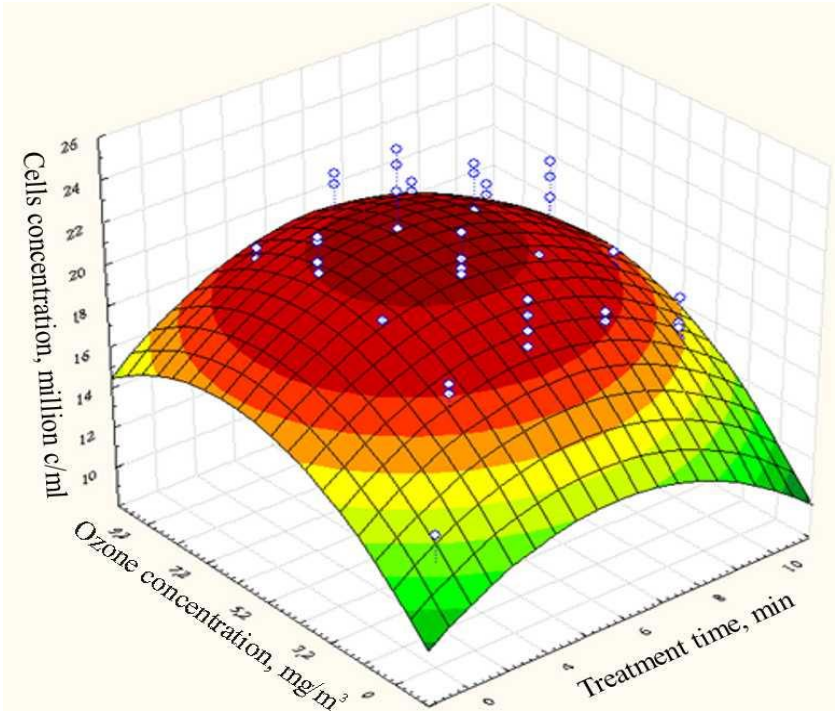

Figure 7. Diagram of the treatment time x_1 and ozone concentration x_2 influence on the chlorella cells concentration in suspension y_1.

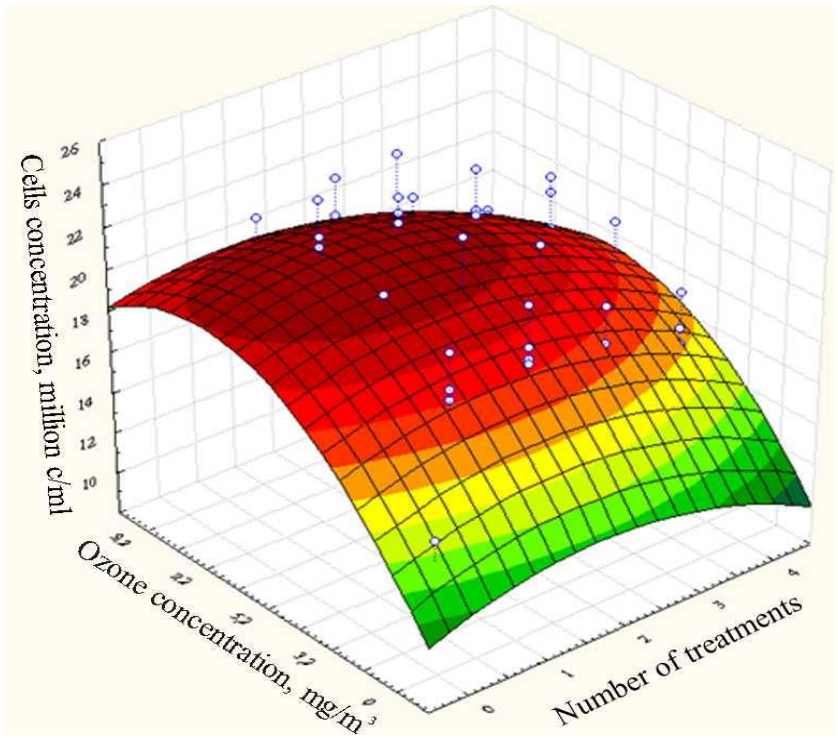

Figure 8. Diagram of the dependence of chlorella cells concentration in suspension y_1 on the ozone concentration x_2 and number of treatments x_3 in four days.

Therefore, the most favorable treatment mode is the mode with the following parameters: ozone concentration – 7.2 mg/m^3 and treatment time 6 minutes. Specified parameters of treatment correspond to chlorella cells concentration in suspension of 24.5 million c/ml, which is 10 million c/ml more, in comparison to the control sample. Using the results of a statistical analysis, multiple regression equation was obtained, which indicates influence of the factors being examined on the independent variable – chlorella cells concentration y_1.

It should be noted that during treatment of the suspension with ozone concentration of 7.2 mg/m^3 , results for chlorella cells concentration were the highest, therefore, in order to find effective ozone influence it is necessary to determine periodicity of such treatment.

For this purpose, diagrams of the dependence of chlorella cells concentration in suspension y_1 on the ozone concentration x_2 and number of treatments x_3 in four days were constructed (Figure 7 and 8).

Analysis of the obtained surfaces indicates, that with ozone concentration of 7.2 mg/m^3 increase of the chlorella cells concentration can be observed if number of treatments goes up from 1 to 2 in four days. Increase in a number of treatments gives decrease of the chlorella cells concentration in suspension. Therefore, it is worthwhile to perform a treatment with periodicity 2 times in four days.

4. Conclusion

Obtained regression equation allows to determine degree of influence of every factor on the process of chlorella microphytic alga stimulation.

Conducted regression analysis of the experimental data indicated that being examined parameters (concentration, treatment time, number of treatments) in 85% of the cases influence on the increase in chlorella cells concentration.

Based on the results of the experimental research, treatment modes of a chlorella strain suspension Chlorella vulgaris IGF S – 111 were determined, which allows to double the increase in productivity of a propagator:

- ozone concentration – 7.2 mg/m^3;
- treatment time – 6 minutes;
- number of treatments – 2 times in four days.

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