UDC 662.767.3

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EFFECT OF SULPHUR COMPOUNDS ON CULTIVATION PROCESS OF MICROALGAE *CHLORELLA VULGARIS*

Робота присвячена дослідженню впливу концентрації сполук сульфуру на процес культивування мікроводорості *Chlorella vulgaris* з метою застосування як джерела поживних речовин газових викидів промислових підприємств. Аналізуються зміни pH культурального середовища і приріст біомаси за одночасного введення CO_2 та H_2SO_4 або Na_2SO_3. Показано, що збільшення концентрації Сульфуру в середовищі до 0,44 г/л за використання сульфіту натрію підвищує продукування біомаси на 15% і не викликає відхилення значень pH від оптимального діапазону.

Ключові слова: мікроводорості, газові викиди, приріст біомаси, сполуки сульфуру, вуглекислий газ.

The article deals with the investigation of the influence of sulfur compounds concentration on the cultivation of microalgae Chlorella vulgaris for the purpose of future use of flue gases as a nutrient source. The alteration of culture medium pH and microalgae biomass production under simultaneous injection of CO_2 and H_2SO_4 or Na_2SO_3 are studied. It is shown that the enhancement of sulphur concentration to 0.44 g/L by using Na_2SO_3 increases the biomass growth by 15 percent and does not cause deviations from the optimal pH range.

Key words: microalgae, flue gases, biomass production, sulfur compounds, carbon dioxide.

Introduction. Since society encountered rapid inventory shortage of fossil fuels and negative environmental effects of their usage, it has become urgent to produce energy from renewable raw materials, first of all, from different kinds of biomass, such as wood, agricultural industrial and food crops. Such biofuels, called as first generation biofuels, compete for cultivable lands, and their production depends on weather conditions. Thus, the development of biofuels of second and third generations is an actual problem. Biofuels from microalgae is a kind of such biofuels, because of the cultivation of microalgae can be implemented in closed systems – photobioreactors, which are placed on non-agricultural lands [1].

Comparing to traditional oil crops, microalgae usage for biofuels production has several advantages due to the opportunity to control qualitative and quantitative composition of desired products as a result of different metabolism modifications. The last one can be achieved by variation of nutrient medium composition or of parameters of cultivation process. Moreover, microalgae can grow on various substrates, so the use of flue gas from industrial enterprises as a nutrient source is possible. Additionally, such approach will help to reduce anthropogenic air pollution. Today existing technology of using flue gases for microalgae cultivation include preliminary gas clearing, that affects the biofuel production cost. Besides biofuels, microalgae is a good source of others biologically active compounds, selling of which considerably reduces the final biofuel price [2–3].

Light, water and inorganic nutrient compounds are required for providing of cell growth and reproduction. Among nutrients, the main role for microalgae play Carbon, Nitrogen, Sulphur, Phosphorous and some microelements, such as Na, K, Mg, Fe, Zn, etc. Flue gases can be the source of such nutrients because their contain carbon dioxide CO_2, sulphur SO_x and nitrogen NO_x oxides. Sulphur dioxide is one of the most hazardous air pollutants, and SO_2 emissions during coal combustion are nearly 120 thousand tons per year. In addition to this, sulphur dioxide is the main cause of acid rains which have negative impacts on plant world and lead to metal construction corrosion.

Thus, the investigation of microalgae cultivation process under usage of flue gases is an actual issue. In order to avoid toxic effects of gas oxides, it is possible to use the mixture of flue gases with air (under the variation of gas composition) [4]. SO_2 and NO_2 in the water solution interact with air oxygen or oxygen generated by microalgae in photosynthesis process, and turn into the form of SO_3^{2-}, of SO_4^{2-} and of NO_3^-, that are suitable for microalgae consumption [5].

Periodical bubbling of culture medium with gas mixture with higher content of CO_2 (10 – 15 volume %) intensify the microalgae cell division in 6 times, positively affects biomass production and stimulates microalgae lipid accumulation, that can be used in biodiesel production [4].

Sulphur is a structural element of proteins, enzymes, peptides, some aminoacids, vitamins and coenzymes in microalgae cell and is also a component of many others organic cell compounds. Physiological role of sulphur in *Chlorella* sp. is connected with cell division process, protein metabolism and fatty acid synthesis. Cell sulphur demand can be provided by inorganic SO_4^{2-} introduction to the culture medium. Sulphur flux into the microalgae results from active transport; the process requires light and is temperature-sensitive [6]. Thereby, injection of SO_2 with bubbling air, which use as a nutrient by cell, enhance the microalgae biomass production. Sulphur deficiency in the culture medium is a stress factor for cells and leads to lipid accumulation and to disorder of cell division [6]. At the same time, high concentrations of SO_x and CO_2 in the bubbling air decreases medium pH that has an inhibitory effect on the culture growth [3]. Therefore, the investigation of microalgae growth process under simultaneously injection of sulphur oxides and of CO_2 to the culture medium is of great importance.

The object of this study was to investigate the influence of sulphur compound on biomass production of microalgae *Chlorella vulgaris.*

Determined tasks:

1. Study the effects of culture medium pH alteration on *Chlorella vulgaris* growth under simultaneous introduction of sulfuric acid and CO_2.
2. Study the effects of sulphur compounds in various concentrations on biomass production of *Chlorella vulgaris.*

Materials and methods. The microalgae culture *Chlorella vulgaris* ACKU 531-06 was taken from collection of Taras Shevchenco National University of Kyiv. The medium used for cultivation was Gromova medium No.6 consisting of (g/l): KNO_3, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; KH_2PO_4, 0.2; $NaHCO_3$, 0.2; $CaCl_2$, 0.15; $NaBO_2 \cdot 4\ H_2O$, 2.65×10^{-3}; $MnSO_4 \cdot 4H_2O$, 1.81×10^{-3}; $ZnSO_4 \cdot 7H_2O$, 0.22×10^{-3}; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 1.0×10^{-3}; $Ca(NO_3)_2 \cdot 4H_2O$, 2.0×10^{-5}; $CuSO_4 \cdot 5H_2O$, 7.9×10^{-5}; $FeSO_4 \cdot 7H_2O$, 9.3×10^{-3}; Na_2ЕДТА, 1.0×10^{-2}. The microalgae culture was grown in photoreactors (1-L transparent glass cylinders, diameter 0.04 m, length 0.25 m). The working volume of each reactor was 250 ml. During the cultivation, *Chlorella vulgaris* produces antibiotic chlorellin that prevents medium contamination with others microorganisms, so the asepsis maintaining isn't obligatory [6].

As a modeling medium that contains sulphur compounds, Na_2SO_3 (chemically pure) and H_2SO_4 in the concentration rage (0.2 …0.5 vol. %), that corresponds to average sulphur oxide content in flue gases, were used.

The studies were carried out in three replications. The sulfuric acid was chosen based on the fact that sulphur oxide SO_2 oxidizes in the solution and turn into the form of SO_4^{2-}. Sodium sulfite was used because of sulfite-ions SO_3^{2-} form during the SO_2 water dissolution; additionally, it may be possible to inject alkali in order to neutralize protons generated during culture medium aeration with CO_2 and other acid oxides.

For the supplementation of microalgae culture with carbon source CO_2 obtained in the alcoholic fermentation process was used. Air mixture with higher CO_2 content (10 vol. %) was used for bubbling, supply interval – 12 hours, duration time – 5 minute, flow rate – 0.025 m^3 per hour.

pH value was measured by ionometer И-160 МИ (Russia).

Observation and culture clearness control were carried out using light microscopy (Carl Zeiss Primo Star Microscope, Germany); image magnification from 40x to 1000x for visual observing.

Microalgae were cultured at 30 ± 2°C temperature into the thermostat (ILM Labor, Germany). Light source: three luminous tube lamps, 8 W (Sylvania Groux "Аква свет", Russia); illumination interval – 12 hours light, 12 hours dark.

Table 1

Sulphur compounds concentration and initial pH value of culture medium

No. sample	C (H_2SO_4), mmole/L	C (Na_2SO_3), mmole/L	pH_{initia}
Control	–	–	6,35
1	0,29	–	6,15
2	0,57	–	5,3
3	0,85	–	5,15
4	1,13	–	5,0
5	–	0,5	7,9
6	–	0,85	8,0
7	–	1,2	8,2
8	–	1,7	8,3

Cell quantity *N* per unit of volume was counted using hemocytometer (standard procedure) [8].

Specific growth rate μ was determined as indicated in Eq. (1), [9]:

$$\mu = (\ln N_t - \ln N_0)/t, \quad (1)$$

where N_t – number of microalgae cells in unit of volume at time, *t*; N_0 – number of microalgae cells in unit of volume at the beginning of cultivation; *t* – cultivation time, days.

Results and discussion. *Effect of sulfuric acid on growth and biomass concentration of the microalgae culture*

The microalgae *Chlorella vulgaris* can grow at wide pH range from 5.0 to 8.5 [6]. Initial pH value of the control sample was 6.35 (table 1). Figure 1 illustrates time-course change of medium pH under additional injection of H_2SO_4. Initial decreasing pH values in the control sample and the sample No.1 is caused by medium bubbling with carbon dioxide. Stabilization pH value during the next six days of cultivation indicates the constant culture grown and the consumption of CO_2 as a carbon source. Increasing pH value after six days of cultivation is caused by enhancing of biomass production under constant amount of CO_2 supply and log-phase of culture growth. Sulphur acid introduction doesn't affect the pH value of culture medium during the microalgae grown (2 – 12 days).

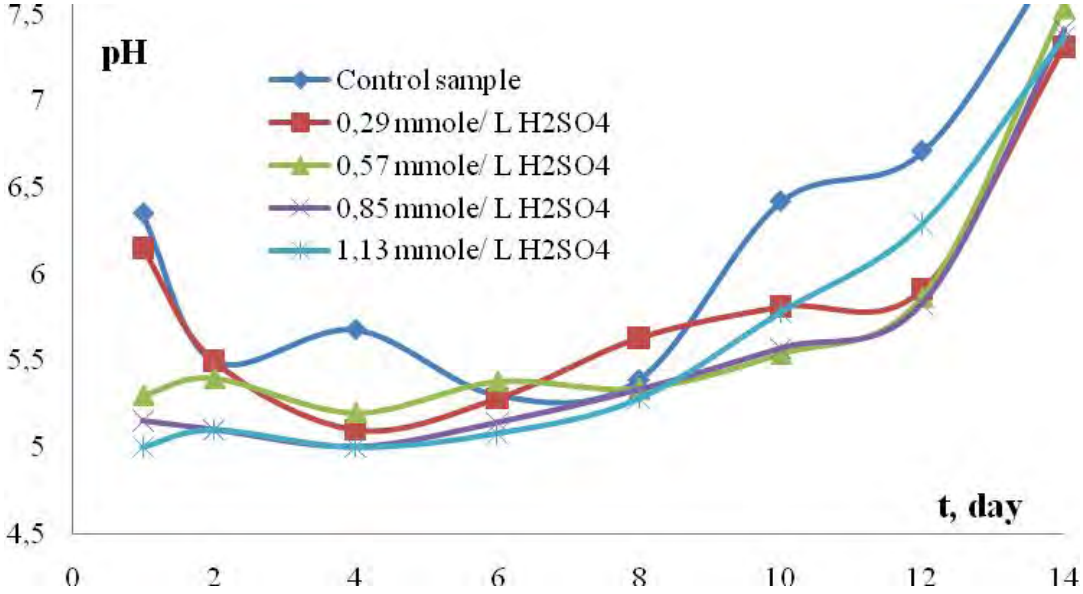

Fig. 1. Change of pH value during the cultivation of Chlorella vulgaris on the culture mediums with various H_2SO_4 concentrations

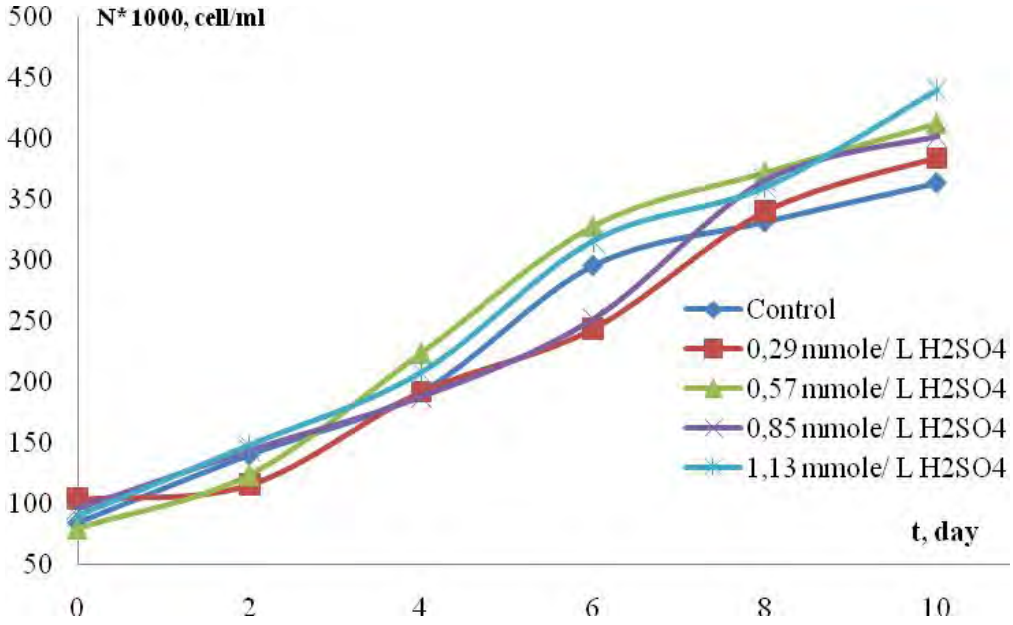

Fig. 2. Time-course microalgae biomass production during the 10-days cultivation on the culture mediums with various H_2SO_4 concentrations

Effect of sodium sulfite on growth and biomass concentration of the microalgae culture

The increasing of pH value with the introduction of sodium sulfite to the culture medium (table 1) is occurs due to the hydrolysis process and to the formation of hydroxide-ions OH^- in the solution. The pH value decreases under stable periodic CO_2 injection to the medium (figure 4) because of the biomass amount, presented in the photoreactor, is not enough for CO_2 total consumption as a carbon source. The increasing of pH value after 8 days of cultivation is accounted for biomass generation, and, respectively, for enhancement of CO_2 consumption.

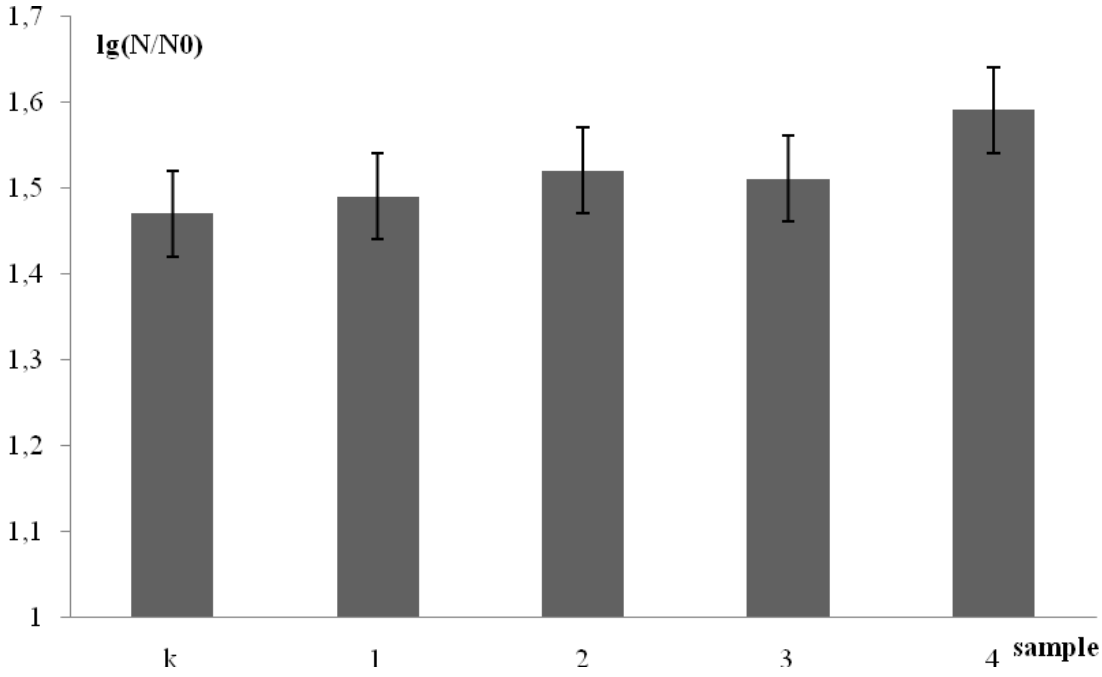

Fig. 3. Microalgae biomass production during the 10-days cultivation on the mediums with various H_2SO_4 concentrations: c – control, without addition of H_2SO_4; 1 – 0,29 mmole/L H_2SO_4; 2 –0.29 mmole/L H_2SO_4; 3 – 0,85 mmole/L H_2SO_4; 4 – 1,13 mmole/L H_2SO_4

Figures 5 and 6 illustrate the culture grown and microalgae biomass production under addition of sodium sulfite. Effects of sulphur containing salts on the cell grown and division could be caused by either a cation or by an anion. Na^+-ions affects the alteration the wide range of macromolecules and their hydrate shells, and, accordingly, change macromolecule structures and physiological activities. Increasing of Na^+-concentration leads to enhancement of salinity in the culture medium and might be an inhibitory factor for microalgae grown because of affect the osmotic regulation process. In this case, the accumulation of small molecules of osmoregulating compounds (such as poluatomic alcohols, glycerol, phitol, low-molecular fatty acids) occurs. Previous study [10] shows that injection to the culture medium additional amount of Na^+-ions (2.5 g/L) positively affects microalgae biomass production. Introduction of additional amount of both Na^+ and SO_3^{2-} to 1.7 g/L salt concentration has a positive effect on culture grown (figure 5 and 6). Biomass production in these samples increases by 15 % (figure 6) comparing to control sample. At the same time, cell doubling in log-phase in the case of sodium sulfite usage occurs in half the time versus in the case of sulphur acid usage or of the Gromova medium No.6 (figure 2 and 5). This can be explained by the fact that SO_3^{2-} oxidize to SO_4^{2-} that is an assimilation form for microalgae, and, correspondingly, the amount of consumed sulphur also increases. Simultaneously, the pH values in these samples remain at optimal level for the grown of microalgae *Chlorella vulgaris*.

Hereby, introduction of additional amount of sulphur to the culture medium positively affects *Chlorella vulgaris* biomass production because such approach leads to stimulation of protein and fatty acid syntheses. The amount of enzymes, that play important role in the redox and energy processes (the bound formation between SH-group of enzyme and NAD and FAD coenzymes, regulation of ATP- synthetase, etc.) also increases. Moreover, various vitamins and cofactors in microalgae cell contain sulphur (e.g. biotin, thiamin, coenzyme A, glytation, lipoic acid, etc.), so regulation of different cell metabolism pathways is also possible.

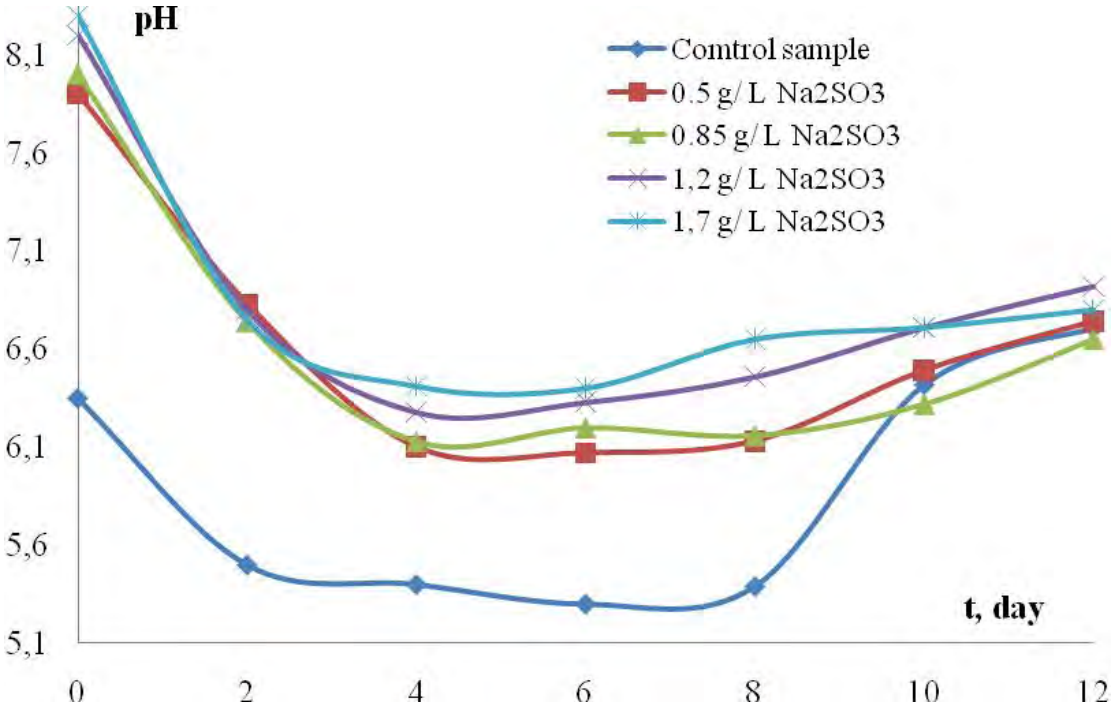

Fig. 4. Change of pH value during the cultivation of Chlorella vulgaris on the culture mediums with various Na_2SO_3 concentrations

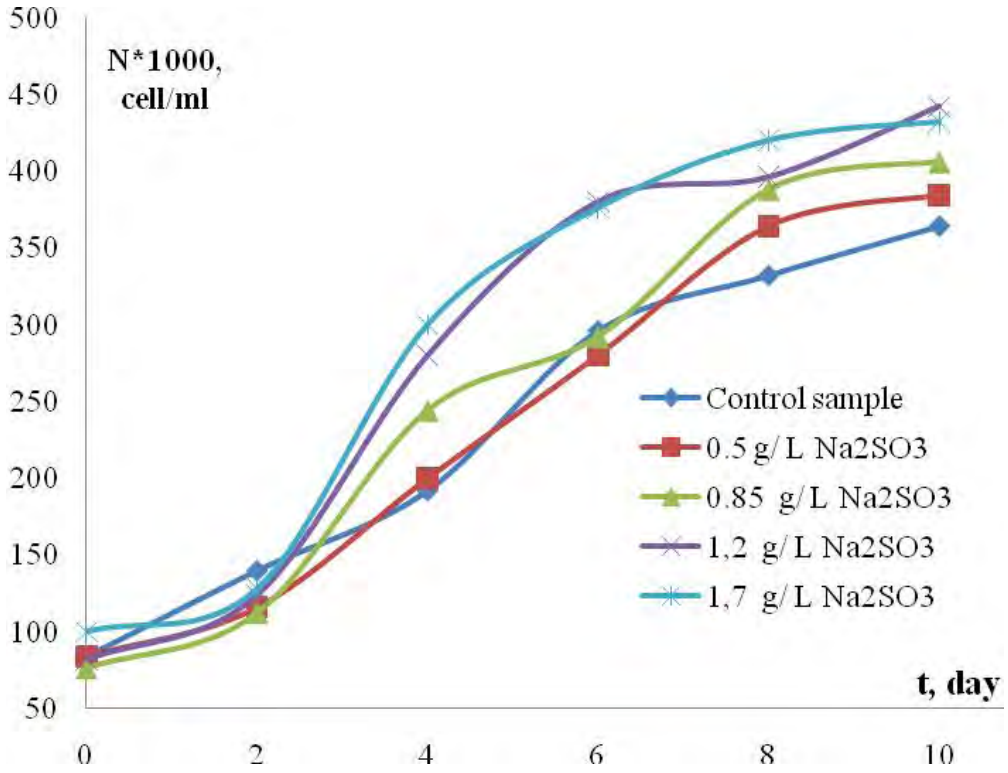

Fig. 5. Time-course microalgae biomass production during the 10-days cultivation on the mediums with various Na_2SO_3 concentrations

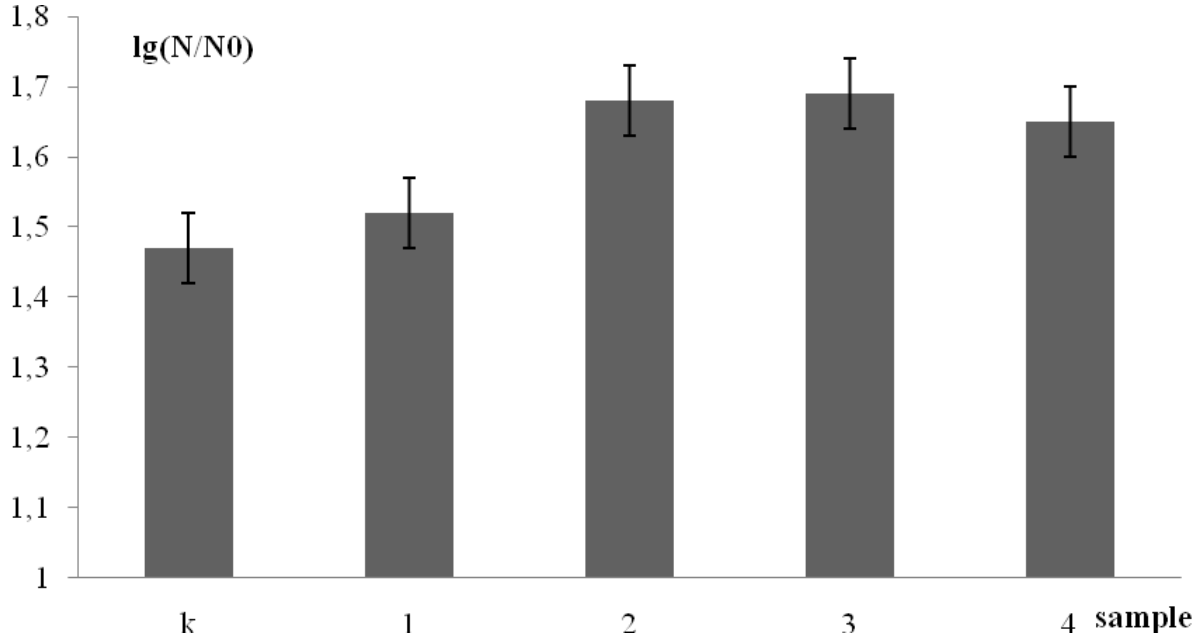

Fig. 6. Microalgae biomass production during the 10-days cultivation on the mediums with various Na_2SO_3 concentrations: c – control, without addition of Na_2SO_3; 1 – 0,5 g/L Na_2SO_3; 2 – 0,85 g/L Na_2SO_3; 3 –1,2 g/L Na_2SO_3; 4 – 1,7 g/L Na_2SO_3

The sulphur introduction to culture medium in the form of sodium sulfite leads to higher biomass production than in the case of sulfuric acid, since the H_2SO_4 addition to the medium provokes the lowering of pH to the critical level. The sulphur introduction in the form of Na_2SO_3 doesn't bring additional protons to culture medium that has a positive effect on microalgae cultivation process under usage of CO_2 as a carbon source. Cell division intensification under additional sulphur source occurs at pH values near 7 that is optimal for *Chlorella vulgaris* and can be achieved by use of sodium sulfite. In other words, introduction additional amount of Na^+-ions to the culture medium for the neutralization of acids, formed due to the aeration with flue gases with higher content of carbon and sulphur oxides, is a positive influence on microalgae biomass grown. What is more, under such approach various osmoregulating compounds, such as fatty acids, are produced; that increases total lipid content in microalgae and is suitable for biodiesel production.

Conclusions

1. As microalgae *Chlorella vulgaris* can grow under wide pH range (5 – 8), the H_2SO_4 introduction in the concentrations corresponding to average sulphur content in flue gas under simultaneous aeration with CO_2 doesn't lead to lowering of culture medium pH below the critical value for microalgae.
2. It is possible to use sodium hydroxide for neutralization of protons formed during the culture medium bubbling with flue gas (gas contains carbon and sulphur oxides); since addition of sodium ions (Na^+ concentration to 2.5 g/L) positively affects microalgae biomass production and fatty acid synthesis.
3. Microalgae biomass production increases by 15 % under enhancement sulphur concentration (0.44 g/L) in the culture medium comparing to microalgae cultivation on the standard (Gromova No.6) medium.

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