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Biogas production from biomass of microalgae Chlorella vulgaris in the presence of benzothiophene sulfone

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Abstract. The possibility of methanogenesis of microalgae *Chlorella vulgaris* biomass under the action of natural and artificially created anaerobic consortia in the presence of sulfur-containing xenobiotic benzotiophensulfone demonstrated. It has been shown that, despite a slight decrease in the concentration of intracellular ATP in the used biocatalysts, the process of methanogenesis proceeded quite effectively. It has been established that the components of hydrolyzate of microalgae *Chlorella vulgaris* biomass, remaining after the extraction of lipids, are excellent substrate for the production of biogas.

1. Introduction

Microalgae biomass has long established itself as a promising substrate for the production of various products under the influence of microbial biocatalysts [1-5]. Its use was also effective as a co-substrate (an additional source of proteins and carbon) in the processes of biocatalytic decomposition of xenobiotics [6].

Currently, the possibility of biocatalytic utilization of sulfur compounds using aerobic bacterial cells, in particular, obtained during the desulphurisation of petroleum products, has been shown. The research in the field of alternative technologies desulfurization during the last 30 years was mainly focused in the development of biotechnological strategies to remove sulfur via a serial of reactions realized under aerobic conditions, known as 4S path-way [7-8]. This path was first described for gram-positive bacteria *Rhodococcus erythropolis* IGTS8 [9], but later it was found in other bacteria [10-11]. This multistage process provides a biocatalytic transformation of heterocyclic sulfur-containing compounds dibenzothiophene through a 4-step sequential education dibenzothiophene-sulfoxide (catalyst - monooxygenase DszC), dibenzothiophene sulphone (catalyst - monooxygenase DszC) and 2-hydroxydiphenyl-sulfinate (catalyst - monooxygenase DszA) 2-hydroxybiphenyl and sulfite (the catalyst - desulfenasa DszB) [9]. Then sulfite is assimilating by its oxidation to sulfate under the action of sulfite oxidoreductase [12]. In addition, the reduced form of flavin mononucleotide is consumed at three stages of oxidation. Its presence in the medium is ensured by the functioning of flavin reductase (DszD) [13].

The main disadvantages of the 4S path-way are the duration of its implementation even at the stage of transition to dibenzothiophensulfone (up to 1 month) and low yields of each intermediate product due to the inhibitory effect of sulfur-containing compounds on the catalytic activity of cells. It is known that the formation and accumulation of sulfones leads to inhibition of whole biocatalytic oxidation of sulfur-containing compounds [14]. Genetic modification and immobilization of cells

allow intensifying 4S path-way, however the optimal solution is still not found. There are a few studies about the effect of such compounds on anaerobic cells metabolism.

Biocatalytic consortia containing sulfate-reducing bacterial strains that can utilize sulfur compounds under anaerobic conditions and its transformation to the same final product as in hydroconversion - to hydrogen sulfide is known [15]. However, such processes are characterized by long time-consuming costs. The speed of the process significantly slows down as hydrogen sulfide accumulates, that imposes restrictions on the possibility of increasing the process speed by increasing biocatalyst concentration.

There are studies describing the effect of other sulfurcontaining xenobiotics, most often dimethyl sulfoxide, on the metabolism of anaerobic cultures [16]. However, specially allocated or adapted anaerobic bacterial cells used at such studies.

As a promising and original solution associated with environmentally safely destruction of sulfones - waste of oxidative desulfurization of petroleum products, it would be possible to consider their biotransformation combined with the methanogenesis processes. However, no data have been presented about the effect of the presence of sulfur-containing xenobiotics (sulfones) at the metabolism of producers during methanogenesis.

So, the aim of this work was to assess the effect of sulfones presence at methanogenesis efficiency of *Chlorella vulgaris* microalgae biomass under action of natural and artificially created anaerobic consortia.

2. Materials and methods

2.1. Microorganisms

Green microalgae cells *Chlorella vulgaris [Beier.] rsemsu Chv-20/11-Пз* were taken from IBCP RAS collections. Wastewater from Ostankino milk processing plant "OOO Ostankinsky Molochny Kombinat" (Moscow, Russia) was used to accumulate *C. vulgaris* biomass. The cultivation was performed in Erlenmeyer flasks (750 mL) containing 100 mL of cultivation medium. The cells were harvested after cultivation using Avanti J 25 centrifuge (Beckman Coulter, USA) at 8,000 rpm for 10 min.

Anaerobic sludge sample formed using the Bogatyr biopreparation (Moscow, Russia). The dry matter (56.4±2.6 g/L), ash content (38.7±1.8 %), and volatile suspended solids (VSS) (34.6±1.6 g/L) were determined according to the published procedure [17-18]. The both acidogenic (1560±30 mg COD/g VSS/d) and methanogenic (80±1 mg COD/g VSS/d) activity of the anaerobic sludge samples were evaluated according to the previously described techniques [17-18]. Nutrition media based on glucose and acetate, respectively, were used in these experiments.

The bacterial strains *Clostridium acetobutylicum* B1787 and *Desulfovibrio vulgaris.* B4053 were obtained from the Russian National Collection of Industrial Microorganisms (www.genetika.ru) for introduction into the methane tank in addition to the anaerobic sludge. The *C. acetobutylicum* strain B1787 was cultivated in the following medium (g/L): glucose – 20; triptone – 10; yeast extract – 5 (pH 6.8). The *D. vulgaris.* B4053 was cultivated in the Postgate medium [19]. Cells were cultivated under anaerobic conditions at 30°C for 1 week. Cultivation of *C. acetobutylicum* and *D. vulgaris* cells was performed under anaerobic conditions in an argon atmosphere at 37°C for 20–24 h and 48 h, respectively.

2.2. *Methods.*

2.2.1. Pretreatment and enzymatic hydrolysis of Chlorella biomass and lipids extraction

Pretreatment and enzymatic hydrolysis of *Chlorella* biomass for its microbial conversion into various products were described in previous work [20]. Biochemical composition of *Chlorella* biomass was done using standard methods described previously [1]. Extraction of lipids from biomass was carried out by the method describe Araujo et al. [21].

2.2.2. *Anaerobic fermentation*

The initial inoculum concentration in batch reactors was 10% (v/v) for suspended anaerobic sludge. The quantity of the artificially immobilized anaerobic sludge introduced into the medium was such as to ensure similar concentrations of sludge biomass in the liquid phase. The anaerobic incubation was carried out at 35°C in all the experiments.

To study the bioconversion of all the substrates to methane the 0.1 M phosphate buffer (pH 7.2) (55 mL) loaded into hermetically sealable vials ("anaerobic reactors", 120 mL). The experiments were performed in triplicate.

A control experiment similar to that described above as usual was concurrently conducted to account for the biogas formation due to the possible lysis of the microbial inoculum [17]. The methane content in the biogas in the experimental control batches was subtracted from that obtained in the corresponding test batches to calculate the methanogenesis efficiency.

2.2.3. Accumulation of biogas and determination of its content.

After 2-5 days we measured the total pressure and biogas concentration in the gas phase of each reactor. Gas measurements were repeated until constant methane content was reached in the gas phase of the reactor.

The content of hydrogen, methane, and carbon dioxide in the gas phase was measured with an LKhM 8 MD chromatograph (Russia) Model 3 with a katharometer (the carrier gas was argon with 20 mL/min flow rate). 2 m long columns were filled with Q porapak [17]. Oven temperature was maintained at 50°C, the retention times of hydrogen, methane, and carbon dioxide were 43, 67, and 82 s, respectively.

2.2.4. Determination of adenosine triphosphate (ATP) concentration

The concentration of intracellular ATP in immobilized cells was determined by the bioluminescent luciferin–luciferase method using cell-containing granules immediately after thawing. For this purpose, granules were weighed (0.15 _ 0.05 g), transferred to dimethyl sulfoxide (1 mL) and allowed to stand at 25ºC for 2 h to extract intracellular ATP [22].

2.2.5. Calculations

The efficiency of methanogenesis (*E*) was calculated using the equation:

$$E = (Q/Q\max) \times 100\ (\%); \quad (1)$$

where Q (mL) is the volume of methane produced in a reactor with a test sample, (equation 2):

$$Q = \{(C/100 \times P_{tot} T_0 V_{gph})/(T_1 P_0)\} \times 1000, \quad (2)$$

where C is the methane content in the gas phase (%); V_{gph} is the volume of the gas phase in the reactor (L); T_0 is the temperature under normal conditions, 273 K; T_1 is the operating temperature in the reactor (K); P_0 is the pressure under normal conditions, 1 atm; and P_{tot} is the total pressure in the reactor (atm),

and Qmax (mL) is the theoretical maximum volume of CH_4 (equation 3):

$$Q\max = (C_I V_{lph}) \times 0.35 \times 1000\ (\text{mL}), \quad (3)$$

where C_I is the initial concentration of the organic substances in the used waste sample, (g COD_{tot}/L); V_{lph} is the volume of the liquid phase in the reactor (L); and 0.35 is the volume of methane produced from 1 g COD at 273 K (L).

The specific productivity of methanogenesis was calculated as the volume of methane formed from 1.0 g of COD under the influence of anaerobic microbial inoculum introduced into the reactor (mL CH_4/g COD).

The data were shown as means of at least three independent experiments ± standard deviation (± SD). Statistical analysis was realized using SigmaPlot 12.5 (ver. 12.5, Systat Software Inc., San Jose, CA, USA). The significant ($p \leq 0.05$) differences between obtained results were estimated by one-way analysis of variance (ANOVA).

3. Results and Discussion

The following experiment was carried out to assess the feasibility of methanogenesis under the action of anaerobic consortia in the presence of sulfones. Benzothiophenesulfone (BTO_2) at a concentration of 0.15 mM was used as the sulfur-containing compound. This concentration was chosen based on the analysis of known data on the decomposition of sulfur-containing xenobiotics by anaerobic bacterial cells. Various combinations of anaerobic sludge, sulfate-reducing bacteria *D. vulgaris* [19] and anaerobic producers of hydrogen, ethanol, cellulases *C. acetobutilycum* were used as anaerobic biocatalysts [1, 3, 5]. These cultures were introduced into artificially created anaerobic consortia to intensify the process of methanogenesis.

It is known that the processes of xenobiotics decomposition proceeds much more efficiently in the presence of additional sources carbohydrates. [23]. Glucose is traditionally used as such carbon source. However, the transition to a cheaper source of raw materials could contribute to the implementation of such developments. It was decided to use hydrolysates of *C. vulgaris* biomass, containing polysaccharides and proteins, as an additional carbon source. Such substrate is a waste of lipid extraction from *C. vulgaris* biomass obtained during wastewater treatment. [3, 4, 24]. As a control similar process was performed using glucose as an additional source of carbon (Figure 1).

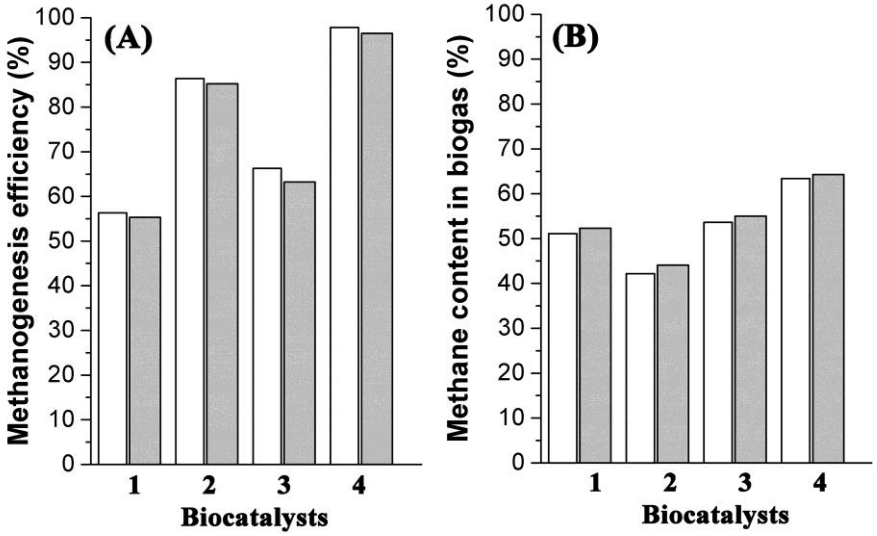

Figure 1. Basic characteristics of the methanogenesis with glucose (white bars) or hydrolysates of *C. vulgaris* biomass (grey bars) and different biocatalysts (1- anaerobic sludge, 2 - anaerobic sludge + *C. acetobutilycum* cells, 3 - anaerobic sludge + *D. vulgaris* cells, *4* - anaerobic sludge + *C. acetobutilycum* cells+ *D. vulgaris* cells) in the presence of 0.15 mM BTO_2

The transition from glucose use to hydrolyzed biomass of microalgae *C. vulgaris* as a carbon source does not reduce the main indicators of methanogenesis, carried out under the action of anaerobic consortia in the presence of sulfones. Obviously it is necessary to create anaerobic consortia, which contain additional cells, allowing intensifying the different stages of a complex biotechnological process with a lot of intermediate enzymatic reactions for the most effective methanogenesis in the presence of sulfones.

Evaluation of the energy status (viability) of anaerobic consortia components (Table 1), indicate that, in general, the concentration of intracellular ATP decreases in all cells used in the process in the presence of BTO_2. The degree of BTO_2 influence at various anaerobic cells is varied. The maximum negative effect from sulfones presence was noted for cells of sulfate-reducing bacteria *D. vulgaris*. At the same time, the presence of BTO_2 at a concentration of 0.15 mM practically did not negatively affect at viability of *C. acetobutilycum* cell (Table 1).

The observed decrease of the specific concentration of intracellular ATP is not a limiting factor for considering the process with the participation of anaerobes as a promising and further study and implementation of methanogenesis in the presence of sulfones, possibly simultaneously with their biological destruction. The decrease in the intracellular concentration of ATP even of anaerobes in the experiment conditions, particularly, may be due to including insufficient concentration of nutrients in the media, because in the control (medium without BTO2) also observed a decrease in the intracellular concentration of ATP compared with the initial level of this parameter.

Table 1. The concentration of intracellular ATP ($\times 10^{-12}$, mol/g biomass) in anaerobic bacterial cells used in methanogenesis of biomass *C. vulgaris* hydrolysate in the presence of BTO_2.

Biocatalyst	Initial	At the end of the process		% reduction from initial	
		without BTO_2	with BTO_2	without BTO_2	with BTO_2
Anaerobic sludge	50.2±2.1	15.4±0.6	1.5±0.1	69.3	97.0
C. acetobutilycum	66.4±3.1	4.2±0.1	4.0±0.1	93.7	94.0
D. vulgaris	27.3±0.3	23.6±0.9	12.1±0.5	13.6	55.7

However, if it is necessary to produce a biotechnological process in the presence of sulfur-containing xenobiotics, then may be it is appropriate to use artificially immobilized microbial cells for reduce negative influence of xenobiotics on anaerobic cells. Such approach has long been positively proven itself in different biocatalytic transformation processes of renewable raw materials, in particular, waste [1-5, 22-23, 25].

4. Conclusion

The possibility of methanogenesis process in the presence of sulfur-containing xenobiotic BTO_2 was demonstrated under the action of natural and artificially created anaerobic consortia. It has been shown that, despite a slight decrease in the concentration of intracellular ATP in the used biocatalysts, in general, the methanogenesis process proceeded quite effectively. It has been established that the components of microalgae *Chlorella vulgaris* biomass hydrolyzate, remaining after the extraction of lipids, are an excellent substrate for the production of biogas.

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References

[1] Efremenko E N, Nikolskaya A B, Lyagin I V, Senko O V, Makhlis T A, Stepanov N A, Maslova O V, Mamedova F and Varfolomeyev S D 2012 Production of biofuels from pretreated microalgae biomass by anaerobic fermentation with immobilized *Clostridium acetobutylicum* cells. *Bioresour. Technol.* **114** 342–348.

[2] Senko O V and Efremenko E N 2017 Highly concentrated populations of *Aureobasidium pullulans* cells in the biocatalytic processes of pullulan production. *Catalysis in Industry,* **9(4)** 344–348.

[3] *Immobilized cells: biocatalysts and processes*. Ed. E. Efremenko (Moscow RIOR) 2018, 499 p.

[4] Maslova O V, Stepanov N A, Senko O V and Efremenko E N 2019 Production of various organic acids from different renewable sources by immobilized cells in the regimes of

separate hydrolysis and fermentation (shf) and simultaneous saccharification and fermentation (sff). *Bioresour. Technol.* **272** 1–9.

[5] Efremenko E N, Stepanov N A, Nikolskaya A B, Senko O V, Spiricheva O V and Varfolomeev S D 2011 Biocatalysts based on immobilized cells of microorganisms in the production of bioethanol and biobutanol. *Catalysis in Industry* **3(1)** 41–46.

[6] Kamal Z M., Nashwa A H., Fetyan A., Ibrahim M A., Sherif E N. 2008 Biodegradation and detoxification of malathion by of *Bacillus thuringiensis* MOS-5. *Aus J Basic Appl Sci* **2** 724-32.

[7] Martínez I, Mohamed M E S, Rozas D, García J L, Díaz E 2016 Engineering synthetic bacterial consortia for enhanced desulfurization and revalorization of oil sulfur compounds. *Metab. Eng.* **35** 46-54.

[8] Nuhu A A 2013 Bio-catalytic desulfurization of fossil fuels: a mini review. *Rev. Environ. Sci. Bio*. **12(1)** 9-23.

[9] Gallagher J R, Olson E S, Stanley D C 1993 Microbial desulfurization of dibenzothiophene: A sulfur-specific pathway. *FEMS Microbiol. Lett.* **107(1)** 31-35.

[10] Kilbane II J J 2006 Microbial biocatalyst developments to upgrade fossil fuels. *Curr. Opin. Biotechnol*. **17(3)** 305-314.

[11] Mohebali G, Ball A S 2008 Biocatalytic desulfurization (BDS) of petrodiesel fuels. *Microbiol*. **154(8)** 2169-2183.

[12] Aggarwal S, Karimi I A, Kilbane II J J, Lee D Y 2012 Roles of sulfite oxidoreductase and sulfite reductase in improving desulfurization by *Rhodococcus erythropolis*. Mol. Biosyst. **8(10)** 2724-2732.

[13] Gray K A, Pogrebinsky O S, Mrachko G T, Xi L, Monticello D J and Squires C H 1996 Molecular mechanisms of biocatalytic desulfurization of fossil fuels. Nature Biotechnol. **14(13)** 1705-1709.

[14] El'kin A A, Grishko V V and Ivshina I B 2010 Oxidative biotransformation of thioanisole by *Rhodococcus rhodochrous* IEGM 66 cells *Appl. Biochem. Microbiol.* **46(6)** 586-592

[15] Kurita S, Endo T, Nakamura H, Yagi T, Tamiya N 1971 Decomposition of some organic sulfur compounds in petroleum by anaerobic bacteria. *J. Gen. Appl. Microbiol.* **17(3)** 185-198.

[16] Lomans B P, Op den Camp H J, Pol A, van der Drift C, Vogels G D 1999 Role of methanogens and other bacteria in degradation of dimethyl sulfide and methanethiol in anoxic freshwater sediments. *Appl. Environ. Microbiol*. **65(5)** 2116-2121.

[17] Gladchenko M A, Kovalev D A, Kovalev A A, Litti Yu V and Nozhevnikova A N 2017 Methane production by anaerobic digestion of organic waste from vegetable processing facilities. *Appl. Biochem. Microbiol.* **53(2)** 242–249.

[18] Kalyuzhnyi S, Gladchenko M, Starostina E, Shcherbakov S and Versprille A 2005 Combined biological and physico-chemical treatment of baker's yeast wastewater. *Wat. Sci. Tech* **52(1-2)** 175–181.

[19] Ismayilov I T, Stepanov N A, Efremenko E N and Abbasov V M 2015 Evaluation of biocidal properties of vegetable oil-based corrosion inhibitors using bioluminescent enzymatic method *Mosc. Univ. Chem. Bull.*. **70(4)** 197–201.

[20] Stepanov N. and Efremenko E 2018 "Deceived" concentrated immobilized cells as biocatalyst for intensive bacterial cellulose production from various sources. *Catalysts* **8** 1–16.

[21] Araujo G S, Matos L J and Fernandes J O 2013 Extraction of lipids from microalgae by ultrasound application: prospection of the optimal extraction method. *Ultrason Sonochem* **20** 95–98.

[22] Stepanov N. and Efremenko E 2017 Immobilised cells of *Pachysolen tannophilus* yeast for ethanol production from crude glycerol. *New Biotech*. **34** 54–58.

[23] Efremenko E N, Lyagin I V, Senko O V, Sirotkina M S and Zavyalova N V 2011 Immobilized heterogeneous biocatalysts for destruction of C-P bond in the hydrolytic products of the organophorous chemical warfare agents *RUDN journal of Ecology and life safely* **1** 61-66

[24] Efremenko E N, Nikolskaya A B, Mamedova F T, Senko O V and Trusov L I 2013 Semicontinuous and continuous process of accumulation of microalgae biomass of *Chlorella vulgaris* cells. *ISJAEE* **2(2)** 44–49.
[25] Senko O, Maslova O, Efremenko E 2017 Optimization of the use of His_6-OPH-based enzymatic biocatalysts for the destruction of chlorpyrifos in soil. *Int.J.Environ. Res. Public Health* **14(12)** 1438.