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Algae-assisted bioelectrochemical system with ammonium, sulfide removal and parallel biomethanation

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In a two-section bioelectrochemical system (BES), the processes of biomethanation of ethanol stillage in the anode zone and oxygenic photosynthesis in the cathodic zone are combined. There is a reduction of ammonium ions and removal of H_2S in the anode zone, and at the same time, there is a positive impact on the process of biomethanation from ethanol stillage in parallel to the anode anaerobic bioreactor. Chemical oxygen demand (COD) reductions ranging from 71.2% to 89.5% were achieved in the 3 BES operating modes studied. The dynamics of the main technological parameters in the bioanode and biocathode area of the BES during continuous operation of the anaerobic bioreactor and photobioreactor (PBR) at a contact time of 10 days is established. Depending on the selected variant of operation of BES - microbial fuel cell (MFC), microbial electrolysis cell (MEC) with 0.6V and 0.9V, a decrease in the ammonium concentration is found to varying degrees, as in the MEC mode with an external electrical voltage of 0.9V the highest degree of 76.5% is reached. At the same time, complete removal of H_2S is found, in the liquid and gas phases in MEC - mode and partially (78 – 84 %) in MFC -mode. The influence of the photosynthetic phases on the electrochemical parameters of MFC was also investigated, with maximum values for power and current densities of 29 W/m^2 and 115 mA/m^2, respectively.

Keywords: biomethanation, bioelectrochemical systems, aerobic photosynthesis, microalgae, microbial sulfate reduction, ethanol stillage, ammonium and sulfide removal.

INTRODUCTION

Environmental issues related to the use of carbon-based fossil fuels and their depletion determine the important role of renewable energy sources in the development of modern civilization [1].

Ethanol stillage is one of the generated waste streams from the brewing industry, with a high risk of pollution of surface water. They are characterized by high acidity (pH: 3.5–4), high organic content (COD: 50÷150 g/L), and nitrogen compounds and sulfates are often found in significant quantities [2]. The formed wastewater from ethanol stillage is difficult to treat, not only because of its characteristics, but also because of its significant volume [3]. On the other hand, the ethanol stillage is successfully utilized in the process of biomethanation [4], but its high sulfate concentrations may make difficult the further treatment of these waters. During anaerobic digestion, sulfates are converted into more toxic sulfides. In general, process inhibition by sulfides does not occur when the ratio COD/SO_4^{2-} in the effluent is > 10 g/g. The inhibition of anaerobic digestion is strong when the ratio COD/SO_4^{2-} is < 0.5 g/g [3].

Ammonium ions in high concentrations also negatively affect biomethanation [5]. Inhibition by ammonium ions can cause more than 30 % loss of methane potential in biogas reactors digesting protein-rich substrates [6]. To alleviate ammonia inhibition, different approaches are applied, but most of them are expensive and of limited application [7].

Anaerobic digestion has established itself as a baseline process for the conversion of organic waste into biogas and is a sustainable approach to its treatment [8]. However, further optimization of anaerobic digestion is limited by insufficient energy recovery and accumulation of inhibitory substances in the medium [9].

In recent years, bioelectrochemical systems (BESs) have proven to be systems that provide attractive opportunities for water treatment from organic and inorganic pollutants, electricity generation, biohydrogen production, bioelectro-methanogenesis, preparation of valuable chemical products, application in biosensors for detection of various pollutants in the aquatic environment, etc. [10].

The BESs also offer further possibilities to improve the anaerobic digestion process - as an after-treatment step for the treatment of the undigested organics (by microbial fuel cell - MFC) in the output stream for further oxidation of the organics with the accompanying energy recovery, and/or bioelectromethanation (by microbial electrolysis cell - MEC) which can be achieved – increasing the methane content in the biogas at the expense of CO_2, overall stabilization of the process, reducing COD at the output, etc. [11].

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An advantage of using BES is the additional possibility of removing the sulfides and hydrogen sulfide formed in the medium in the parallel (with biomethanation) process of microbial sulfate reduction. The produced biogenic H_2S is a mediator in electron transfer, being oxidized on the surface of the anode to elemental sulfur (S^0) and its other forms [12].

The use of algae in microbial fuel cells is of interest due to the possibility of obtaining biomass, energy yield and wastewater treatment for various pollutants [13]. Furthermore, algae-assisted MFCs provide a new, efficient and cost-effective solution to increase the oxygen concentration in the cathodic zone [14], and oxygen is the preferred electron acceptor in the cathode chamber of BES. In a significant part of the previous research, algae are used as biocatalysts in the cathode zone primarily in microbial fuel cells [15]. However, research on the application of algae as a biocatalyst in microbial electrolysis cells is lacking in this regard. The expectation is that applying of additional external voltage (in algae-assisted MEC), will significantly increase the current and power density of the BES and thus achieve a higher rate of pollutant removal in the anode area.

A major objective of the research is to establish the possibility of using algae-assisted BES (in the cathode zone) in combination with biomethanation (in the anode zone), to achieve a higher rate of organic substrate utilization, removal of sulfur and nitrogen compounds, and improve the biomethanation process. An important task in research is to compare two variants of algae-assisted BES – MFC and MEC, in terms of removal of ammonium ions, H_2S, COD and influence on the biomethanation process.

MATERIALS AND METHODS

Substrates, inoculum and enrichment of microbial communities

For the laboratory studies a mixed culture of microalgae dominated by *Scenedesmus sp*., isolated from natural water sources, was used. For the cultivation of the microalgae, the modified culture medium BG11 was used, according to previous studies [16] with the following composition for 1 L – 1.5 g $NaNO_3$, 0.5 g Na_2CO_3, 0.04 g K_2HPO_4, 0.075 g $MgSO_4.7H_2O$, 0.036 g $CaCl_2.2H_2O$, 0.045 g citric acid, 0.0015 g ferric ammonium citrate, 0.045 g EDTA (disodium salt) and 1 ml trace elements solution consisting of 2.86 g/l H_3BO_3; 1.81 g/l $MnCl_2.4H_2O$; 0.222 g/l $ZnSO_4.7H_2O$; 0.39 g/l $NaMoO_4.2H_2O$; 0.079 g/l $CuSO_4.5H_2O$; 0.0494 g/l $Co(NO_3)_2.6H_2O$. The amount of microalgae inoculum was approximately 10 % of the working volume (2.0 dm^3) of the photobioreactor. Cultivation of the microalgae was carried out at room temperature in the range of 24-26 ºC. The oxygenic photosynthesis zone in the combined photobioreactor with the cathode zone of the BES (3) was suspended with air (1.0 dm^3/60s) without further addition of CO_2 (Fig. 1).

The composition of the substrate used (ethanol stillage) for biomethanation in the UASB reactor and the anode zone of the BES is with approximately the same composition under the different operating modes investigated.

Typically, the ethanol stillage is characterized by a high organic load of up to 85-1 10 g/l COD [17], and anaerobic digestion has been found to proceed more steadily at initial COD values below 50 g/l [18].

For the biomethanation process, ethanol stillage from a winery in the village of Svetovrachene, Bulgaria, was used, which was stored in a refrigerator at a temperature of 4 °C. Before being used, the stillage was neutralized to pH 7.5 with NaOH and diluted 4 times. The anaerobic activated sludge was taken from a municipal wastewater treatment plant in Sofia, Bulgaria and was used for inoculation with methanogens. Table 1 shows the main characteristics of the substrate used – ethanol stillage (neutralized and diluted four times).

Table 1. Main characteristics of the ethanol stillage feeding the anaerobic reactor

Parameter	Value
pH	7.4-7.6
COD, g/L	9.45-11.90
SO_4^{2-}, mg/L	163-198
Total Kjeldahl nitrogen (TKN), mg/L	310-325
Dry matter, %	1.67-1.89

Description of laboratory installation

A scheme of the laboratory installation of an integrated bioelectrochemical system (BES) to a UASB (Upflow Anaerobic Sludge Blanket) reactor is shown in Fig. 1. The proposed construction is a combined column photobioreactor (PBR) with a bioelectrochemical system (BES) integrated into its volume. The combined BES-PBR system is in 2 cylindrical plexiglass volumes, "tube-in-tube" type, with the cathode zone having a volume of 2.0 dm^3 (height 400 mm, diameter 100 mm) and the anode zone being an opaque plastic inner tube with a volume of 0.65 dm^3 (height 400 mm, diameter 45 mm). In the volume of the cathode zone (PBR), 4 LED light sources with a wavelength in the range of 400÷700 nm are placed, which provide a light flow

with an intensity in the range of 7600 - 8500 Lx, in the mode - 12h light: 12h dark.

For the anode and cathode in the BES, 2 identical graphite rods with a diameter of 8 mm and a length of 300 mm were used. A cation exchange membrane (CEM), type CMI-7000S (Membrane International Inc.), with an internal diameter of 45 mm, separated the cathode from the anode zones of the BES. In the zone of oxygenic photosynthesis (cathode zone), the possibility of air supply is provided by a pump with a flow rate of 1.0 dm^3/60 s. On the other hand, in the anode area, the possibility of recirculating organic substrate from the UASB – bioreactor for biogas production is ensured by a peristaltic pump with a flow rate of 5 dm^3/h.

The anaerobic UASB biomethanation bioreactor with a geometric volume of 4 dm^3 and a working volume of 3.0 dm^3 was connected to the anode zone of the BES (volume 0.65 dm^3), the liquid phase being continuously recirculated by a peristaltic pump with a flow rate of 5 dm^3/h. The temperature in the bioreactor was maintained in the range of 33-35 ºC by an adjustable electric heater placed at the bottom of the vessel. The incoming substrate (8) is dosed into the reactor at a flow rate of 0.300 dm^3/24h, which provides a contact time of 10 days.

The photobioreactor (resp. the cathode zone of the BES), was daily fed with fresh nutrient medium (BG11) by a peristaltic pump (2) at a flow rate of 200 dm^3/24h, during which a contact time of 10 days was achieved, consistent with the exponential phase of the microalgae growth curve established below.

Analytical methods

The volume of the separated gas was measured using a MilliGascounter "Ritter MGC-1", and the content of CO_2, CH_4, O_2, H_2S and H_2 in the biogas was determined using a portable gas analyzer “Draeger X-am 7000”. At various points in the laboratory installation continuous (online) measurement of dissolved oxygen, pH, voltage, electrical conductivity, temperature and illumination is provided by using Vernier^R BTA sensors and visualization and recording of data through the interface LabQuest^R. The dry matter of the stillage was measured using a Kern DAB moisture analyzer balance.

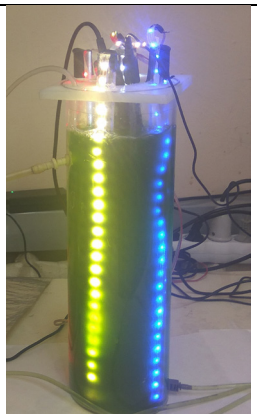
Chemical oxygen demand (COD) was measured with Merck reagents according to APHA (1992) [19]. pH and ORP were measured with a pH/ORP-meter Hanna HI 3220. Sulfate concentration was determined using a spectrophotometric method at λ - 420 nm, using $BaCl_2$ reagent. The concentration of hydrogen sulfide in the liquid phase was measured using Nanocolor test 1-88/05.09 at λ of 620 nm. The concentration of ammonium ions (NH_4^+-N) in the catholyte was determined spectrophotometrically by DR 6000, Nessler Method, Ammonia, 380N HACH.

A Bürker light microscope counting chamber (Boeco^R, BM-800) was used to determine the number of microalgae, as well as a parallel determination of the optical density of the cell suspension during the cultivation of the microalgae at a wavelength of 650 nm and a red filter. Scanning electron microscopy (SEM) was used for scanning the biofilm on the surface of the anode. Before SEM, the samples were fixed with 2 % glutaraldehyde in 0.1 M phosphate buffer overnight in the fridge (4 ºC) and then dried with ethanol. After that, the samples were kept in a desiccator for 48 hours and then coated with a thin layer of graphite.

BES operations and electrochemical analysis

Under the continuous operation of the anaerobic UASB reactor and the photobioreactor (PBR), 4 BES operating modes were investigated (Fig. 1). The first mode is when the electrical circuit of the BES is open (no load between anode and cathode). The second mode is when the BES is operated as a microbial fuel cell (MFC), where a load of 250 Ω is applied, found (below) to be optimal for achieving maximum power density. The third and fourth mode is when the BES is operating as a microbial electrolysis cell (MEC), with the application between the electrodes of 2 different external voltages of 0.6V and 0.9V and a load resistance of 10 Ω.

The electrical parameters of the BES were measured with a Keithley 175 digital multimeter, and a precision potentiometer with a maximum value of 11 kΩ was used for the load resistance. To provide an external voltage source, a stabilized adjustable rectifier type PS-3005D was used when operating BES in MEC mode. The maximum power value, Pmax, was established by constructing polarization curves. The current and power density was calculated based on the geometric area of the electrodes in the anode/cathode chambers and the voltage across the load resistors (R1/R2).



PBR with integrated BES (3)

UASB-reactor for biomethanation (9)

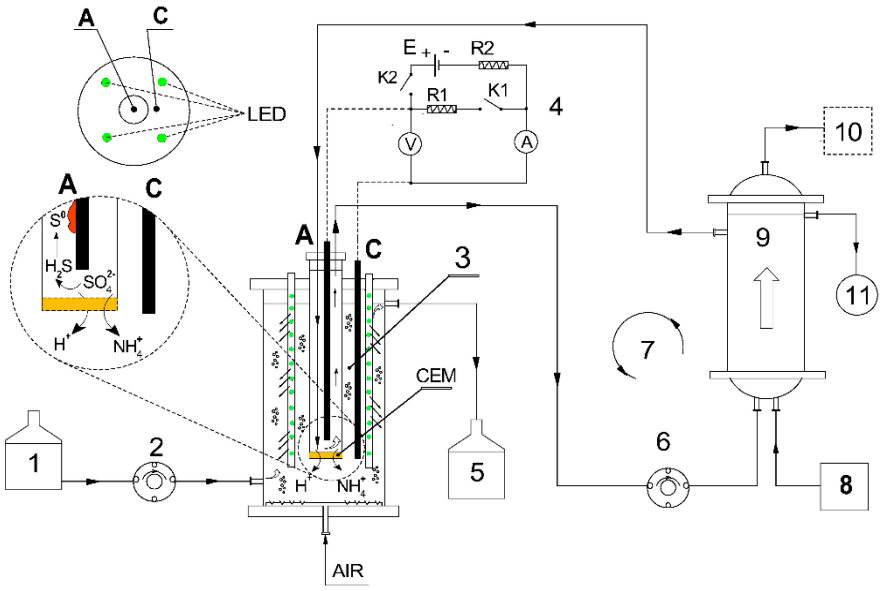
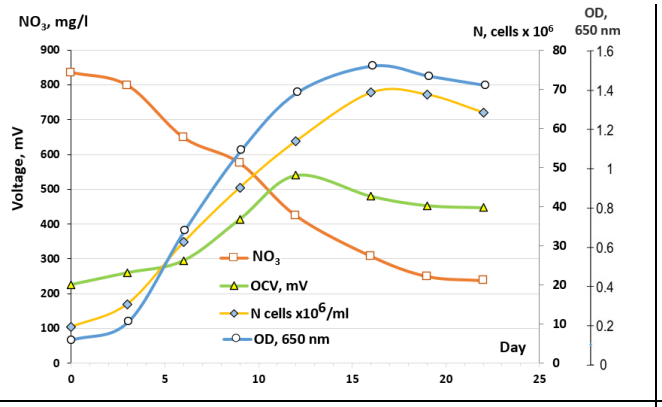
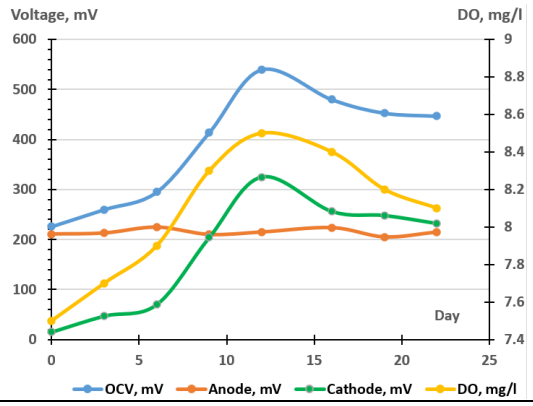


Fig. 1. Scheme of the laboratory installation. 1- input nutrient media for microalgae, 2- dosing peristaltic pump, 3 - integrated BES with a cathodic zone with oxygenic photosynthesis (PBR) and anodic zone with recirculation of substrate for biomethanation, 4- loading circuit of BES, 5- output of PBR (cathodic zone of BES), 6-pump for recirculation between the anode zone of the BES and UASB-reactor for biomethanation, 7-recirculation loop of the anode zone, 8-substrate inlet for biomethanation, 9- UASB-reactor for biomethanation, 10- biogas, 11- outlet spent substrate after AD.



a



b

Fig. 2. Dynamics of nitrate concentration (a), anodic and cathodic potential (b), dissolved oxygen (b) and OCV (b), during the growth phases of algae in the cathodic zone of BES (a).

RESULTS AND DISCUSSION

Cultivation of microalgae and influence of BES electrochemical performance

Initially, to study the influence of microalgae as biocatalysts in the cathode zone, stationary conditions for the biomethanation process were previously established in the contour of the anode zone of the combined BES-PBR system (7). A contact time of 10 days was provided for the feed organic substrate in the UASB reactor (Fig. 1) and stabilization of the process with the uniform release of biogas from 0.92 to 0.94 dm^3/24h (Table 3).

To establish the growth curve of microalgae in the cathode zone of the BES (resp. PBR), in parallel with the measured optical density, the number of microalgae was also determined using a Bürker counting chamber. Cultivation of the microalgae continued for a period of 25 days. Periodically (every 3 days) samples were taken from the culture suspension to determine the number of cells and the optical density (OD). The obtained results (Fig. 2a) show that the stationary phase was reached in about 15 days, with the log phase of growth lasting between 4 and 12 days from the beginning of cultivation. Simultaneously with the growth curve, the nitrate concentration and open circuit voltage (OCV) in the BES were measured in parallel (Fig. 2a). A significant decrease in the nitrate concentration in the medium from 835 mg/l to 238 mg/l over a period of 22 days was found, which is expected given that nitrate is a major food source for microalgae. The highest value of the open circuit voltage (OCV= 540 mV) of the BES was measured at 12 days, which corresponds to the end of the exponential phase of the development of the mixed culture microalgae dominated by *Scenedesmus sp.* Because the anodic potential throughout the period was fairly constant (203 ÷ 224 mV, Fig. 2b), the rise in the OCV value in the BES is obviously due to the growth of the cathodic potential. The reason for this is the increase in the oxygen concentration (from 7.5 to 8.5 mg/l) in the cathode zone due to the development of algae at the end of the log phase (Fig. 2b). Similar results were obtained by Hou *et al.* (2016), who also found a direct relationship between dissolved oxygen concentration in the catholyte and OCV [20].

Also of interest is the establishment of the influence of the phases of photosynthesis of oxygenic microalgae on the bioelectrochemical characteristics of BES. For this purpose, during the period of the log-growth phase (10th day), the power curves and polarization curves of the BES in the mode of operation as MFC were lowered (Fig. 3). In these measurements, the highest values of power and current density were found during the light phase, respectively - 29.0 W/m^2 and 115 mA/m^2 and during the dark phase, respectively -22.6 W/m^2 and 112 mA/m^2. These values were obtained with a load resistance value of – R1 (Fig. 1) in the range of 200-300. The obtained results confirm again the influence of higher oxygen concentrations during the light phase (compared to the dark phase) on the bioelectrochemical characteristics of algae-assisted BES, with analogous data being commented on and observed in other studies [20, 21].

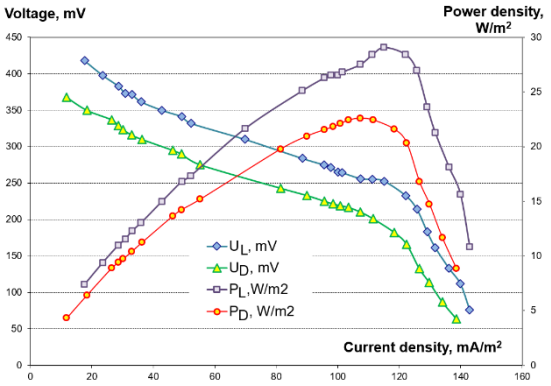


Fig. 3. Power curves and polarization curves measured through the light and dark phases of photosynthesis in the cathodic zone of the BES, during the log-phase (10th day) of the algal development. P_D and P_L, U_D and U_L - power density and voltage through the light (L) and dark (D) phases.

Ammonium, sulfide and COD removal from ethanol stillage by BES system

Along with the process of biomethanation from various organic substrates (including ethanol stillage), a parallel process of ammonification takes place, which breaks down the present proteins, urea and amino acids. This process produces ammonia (NH_3), which is rapidly converted to free ammonium ions (NH_4^+). They are an important source of nutrients for microorganisms and provide buffering capacity in anaerobic digestion processes. The protein degradation process is slow and the released ammonia tends to accumulate in the medium [22]. However, substrates with high nitrogen contents inhibit the process, which can lead to reduced biogas quality and quantity [6].

The integration of the anode zone of the BES to the AD process is one of the approaches to eliminate high concentrations of ammonium ions by their migration through the CEM into the cathodic zone. In the present study, their concentration in the anode zone drops significantly (from the initial value 141÷180 mg/l), and in the cathodic zone, they also decrease, probably due to their absorption by

microalgae and a parallel process of nitrification (Table 2). The removal of ammonium ions in the anode zone (Fig. 4 and Table 2), is most established in the mode of operation - MEC with an external voltage of 0.9 V, reaching up to 25 mg/l (76.5 %), followed by MEC with 0. 6 V – 84 mg/l (53.3 %) and least in MFC mode - up to 105 mg/l (41.7 %). Besides the removal of ammonium ions, there was also a substantial decrease in COD for the 4 modes of operation studied, each characterized by a different current density in the BES. The impact of BES on the COD lowering in the anode zone was significant, with an average current density of 0.34 A/m^2 (for MEC$_{0.9V}$) reaching to 89.5%, respectively, at an average current density of 0.13 A/m^2 (for MEC$_{0.6V}$) - 80.4% and at a current density of 0.05 A/m^2 (for MFC) – 71.2 % (Fig. 4). For comparison, in the mode of operation without BES, the COD reduction in the system is in the range of 45÷55 %. Similar results were obtained by Zhang *et al*. (2019), where when treating swine wastewater in the anode chamber of MFC (a combined system with a photosynthesis process in the cathodic zone with *Chlorella vulgaris*), 85.6 % ammonium removal and up to 83.1 % reduction in total organic carbon (TOC) were reached in 24 days [21]. The presence of high concentrations of sulfates in the organic substrate subject to anaerobic digestion makes the biomethanation process difficult. Methanogens under these conditions compete with sulfate-reducing bacteria (SRBs) for the carbon source, and the H_2S produced, in addition to being corrosive and toxic, also has an inhibitory effect on AD and degrades biogas [23].

By introducing the BES process-based heterotrophic microbial sulfate reduction in the anodic zone, it is possible to realize oxidation on the anode surface of biogenic H_2S to elemental sulfur (and other forms - Fig. 1) and thus, one can reduce the H_2S content in the medium and improve the composition of the obtained biogas [24]. The studies found complete removal (100 %) of H_2S in the gas phase (Table 3) in both MEC modes and a significant decrease (78-84 %) in MFC mode. About the liquid phase (Table 2), complete removal of H_2S and sulfates was also observed in MEC mode with 0.9V external voltage and partly in MFC mode with up to 70 % concerning sulfates and up to 45.3 % reduction of dissolved H_2S.

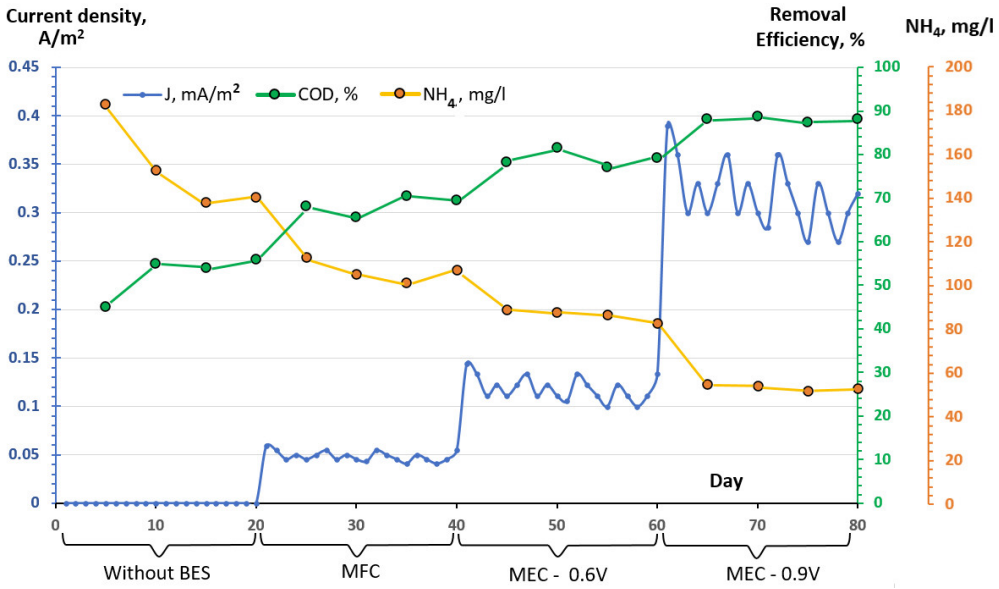


Fig. 4. Dynamics of COD and ammonium ion concentration in the anode chamber, at different current densities, for 4 modes of operation of BES.

Table 2. Main parameters of the liquid phase in the anode zone at 4 modes of the BES.

Parameter	Without BES	MFC	MEC with 0.6V	MEC with 0. 9V
pH	7.4 - 7.8	7.6-8.0	7.7 - 8.1	7.8 - 8.2
COD input, g/L	9.88 – 11.55	9.45 – 11.61	10.20 – 11.90	10.15 – 11.80
COD (anode), g/L	5.48 - 6.69	2.98- 3.67	2.14- 2.49	1.20- 1.40
NH_4^+(anode), mg/L	141-180	105-116	84-90	25-28
NH_4^+(cathode), mg/L	3- 7	10- 15	17- 25	27- 35
SO_4^{2-} , mg/L	144. 3	43. 2	<1	<1
H_2S (in liquid), mg/L	22.5- 21. 6	15.4- 12. 3	5.3- 4. 1	<1

Table 3. Main parameters of the biogas released from the UASB reactor in 4 modes of BES.

Parameter	Without BES	MFC	MEC with 0.6V	MEC with 0. 9V
Daily biogas yield, $dm^3/24h$	0.91-0.93	0.88 – 0.92	0.89- 0.91	0.87-0.90
CH_4, %	59-61	69-71	72-74	72-75
CO_2, %	21-24	18-20	17-19	16-18
H_2S, %	0.041- 0.044	0.009- 0.007	0	0
H_2, %	0.9- 1.0	0.42- 0.45	0.34- 0.37	0

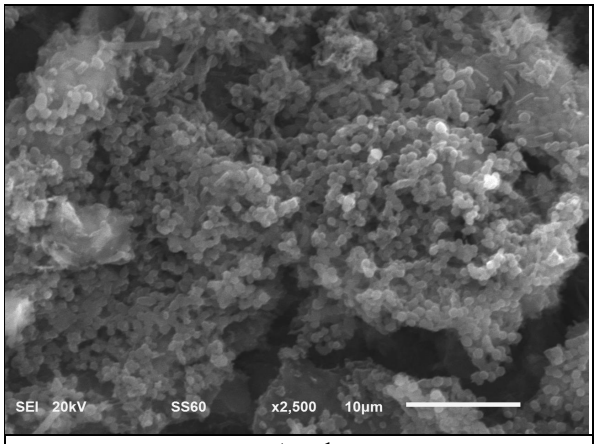
In these results, it is also important to note the pH change during the investigated BES modes (Table 2). In this respect, a slight increase in the pH value is noticeable, the process stabilizing in the range pH = 7.8-8.2 in the $MEC_{0.9V}$ mode. In this regard, the distribution of sulfur forms at different pH values of the environment is known, where under reducing conditions, sulfur can be present in the form of H_2S (gas), HS^- and polysulfides [25]. Therefore, at such pH values, it is logical to expect hydrogen sulfide in the liquid phase to be represented mainly in the form of HS^- - ion.

The integration of algae-assisted BES to AD at the same time leads to an improvement of the quantitative and qualitative composition of the obtained biogas compared to a standalone AD process (Table 3). For example, the daily biogas yield for the entire period (of 80 days) of the experiment was relatively uniform and ranged from 0.88 to 0.93 $dm^3/24h$ in all studied variants, and in terms of qualitative composition – an increase of the CH_4 content to 72-75 % was found in MEC mode with 0.9V, and for comparison in a standalone AD process, CH_4 reaches 59-61 %. The complete removal of H_2S from the biogas when operating BES in the mode of MEC is not to be underestimated.

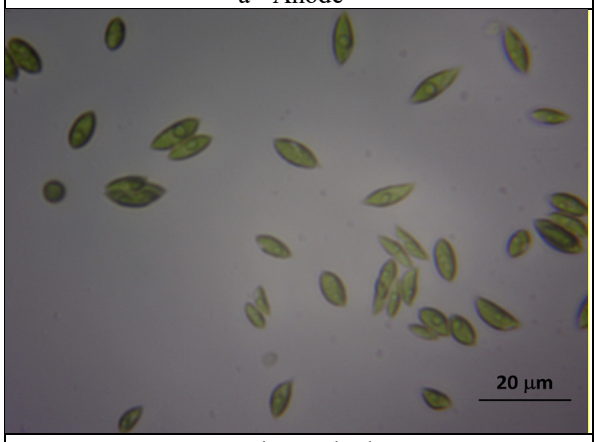
Fig. 5a shows photographs of SEM images of methanogenic bacteria attached to the surface of the graphite anode. The image shows the colonization of mostly coccoid bacteria measuring 1-1.5 μm. In the cathode zone (Fig. 5b) the culture suspension of microalgae was studied with a light microscope, where a mixed culture of microalgae dominated by *Scenedesmus sp*. with a cell size of 8- 12 μm was observed. The observations were made at the end of the 80 days of the experiments carried out in BES operation mode as MEC with 0. 9V external voltage.

CONCLUSION

In the present study, the possibility of using algae-assisted BES in combination with a biomethanation process was demonstrated to achieve a higher degree of utilization of the organic substrate (ethanol stillage), removal of sulfur compounds and ammonium ions and improvement of the composition of biogas.



a - Anode



b - Cathode

Fig. 5. Morphological characteristics of attached on the anodic surface (Fig.5a) methanogens (by SEM) and light microscope image (Fig.5b) of a culture suspension of microalgae dominated by *Scenedesmus sp.*

The dynamics of the main technological parameters in the bioanode and biocathode area of the BES during continuous operation of the UASB reactor and photobioreactor (PBR) with a contact time of 10 days were determined. When comparing 4 different operating modes of the combined algae-assisted BES-AD system - without BES, MFC, MEC (0.6V) and MEC (0.9V), the highest removal rates of ammonium ions (76.5 %), H_2S (100 %) and COD (89.5 %) were found at $MEC_{0.9V}$ mode. The

qualitative composition of the obtained biogas was also improved, reaching up to 75 % CH_4 content. The influence of the phases of photosynthesis on the electrochemical parameters of BES was also established, with maximum values for power density and current, respectively - 29 W/m^2 and 115 mA/m^2.

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