INTEGRATION OF MICROBIAL FUEL CELLS IN A SYSTEM FOR BIOMETHANATION AND PHOTOSYNTHESIS

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ABSTRACT

In the ethanol stillage biomethanation system a microbial fuel cell (MFC) is integrated in the anode area when combined with a process of aerobic photosynthesis of microalgae in the cathode zone. The dynamics of the main technological parameters in the area of the bioanode and biocathode of MFC has been established. Elimination of $H_2S$ from the composition of biogas has been achieved in the integration of the fuel products by achieving a higher degree of mineralization of the organic substrate under the load of fuel products compared to the modes of operation without electric load. The dynamics of the cathode potential during the different phases of photosynthesis has been established in the biocathode zone. Maximum values for power and current densities were achieved – 27.4 mW m$^{-2}$ and 68 mA m$^{-2}$, respectively, during the Log phase of the cultivation of oxygen microalgae.

Keywords: biomethanation, microbial fuel cell, aerobic photosynthesis, microalgae, microbial sulfate reduction, ethanol stillage.

INTRODUCTION

In recent years, non-renewable energy sources are disappearing or reducing and alternative renewable energy sources are searching for. They must be environmentally friendly, cost-effective and not to leave a carbon footprint. Aiming to solve the energy crisis and environmental pollution, bioelectrochemical systems (BESs), in particular microbial fuel cells (MFCs), serve as alternative sustainable energy source.

Anaerobic digestion (AD) is a well-known technology widely used for the treatment of wastewater. MFCs can mainly be advantageous as a buffering system to maintain and recover the AD process whenever AD suffers from inhibition for example, from the presence of a large amount of volatile fatty acids (VFA) in the medium [1]. Treatment of AD effluent in MFC could lower the inhibitory concentration of ammonium. MFCs can be integrated with the existing wastewater treatment system to harvest clean electricity [2]. MFCs can be utilized as a processing unit after the primary treatment or after the anaerobic digestion (AD) process or even as a stand-alone process to remove the organic compounds [3]. The integrated systems may tackle harsh problems and often become more effective than stand-alone systems [4]. The advances made on the MFC integrated wastewater treatments, especially the coupling of MFCs to the anaerobic treatment system, appear to be promising in terms of improvement in treatment efficiencies and resource recovery.

MFC integrating with microalgae at the cathode chamber have ability to provide an oxygen rich environment and to remove CO$_2$ from the cathode compartment through the photosynthetic activity of algae [5 - 7]. In this context, thus MFC integrating with anaerobic digestion at the anodic chamber is a suitable system for overcoming carbon limitation conditions in algae-based treatment systems without the need for external CO$_2$ supply. Produced at the anode CO$_2$ could be diverted to the cathode chamber to enhance microalgae growth.

There is a large potential for the development of algae based cathode MFC systems for wastewater treatment. Integration of MFC in a system for biomethanation and photosynthesis operated at closed circuit showed
greater biomass concentrations vs. open circuit conditions - roughly between 1.5 and 3 times higher biomass concentrations [8].

Gajda et al. (2013) found that oxygen production by photosynthetic organisms can increase energy production by 42 % [6]. Oxygen depletion in a water-based cathode can be avoided by the use of photosynthetic biocatalysts, thus ensuring stable operation of the MFCs. The authors conclude that the photosynthetic biofilm seems to be a good alternative biocatalyst for the actual application of MFC as sustainable energy harvesters and at the same time presents the possibility of carbon fixation.

Biotecnologies can offer a low cost and environmentally friendly alternative to biogas upgrading. For example, microalgal-based CO₂ fixation, enzymatic CO₂ dissolution, fermentative CO₂ reduction and digestion with in situ CO₂ desorption have consistently shown CO₂ removals of 80 % - 100 % and CH₄ purities of 88 % - 100 %, while allowing the conversion of CO₂ into valuable bio-products and even a simultaneous H₂S removal. Removal of H₂S higher than 99 % is typically reported in aerobic and anoxic biotriclet filters, algal-bacterial photobioreactors, and digesters under microaerophilic conditions [9].

Combining anaerobic digestion with bioelectrochemical system allows the development and optimization of hybrid systems due to their complementary character. Hydrogen sulphide is an inhibitory component in AD and a corrosive gas. High potential abiotic oxidation of H₂S on the anode can yield elemental sulphur that can be re-oxidised to sulphur oxyanions or polysulphide, and these compounds can subsequently function gain as an electron acceptor for biological oxidation of organics [10, 11].

Liu et al. (2017) compared the performance of a single- and a cation-exchange membrane-equipped two-chamber BES-AD system at thermophilic conditions [12]. The results demonstrate that an active glucose-fed thermophilic anaerobic sludge could readily (< 3 days) increase biogas production in both reactor configurations by inserting a carbon electrode poised at − 0.8 V (vs. Ag/AgCl). After 3-week operation, the biogas production rates from both BES reactors decreased due to VFA accumulation. Only the two-chamber configuration could enable methane enrichment (98 % CH₄ v/v) in biogas. In other study, CH₄ yield was increased by 9.4 % due to the combination of two reactors and electroactive activity of microbial consortium [13].

The aim of this study is to establish the possibility of upgrading the biomethanation process by integration of MFC in the anode area combined with a process of aerobic photosynthesis of microalgae in the cathode zone. The main expected positive effects for the biomethanation process by integration of MFC are a higher degree of decomposition of the organic substrate, reduction of CO₂ and H₂S in biogas by parallel electricity generation.

**EXPERIMENTAL**

**Laboratory installation of an integrated microbial fuel cell in a system for biomethanation and photosynthesis**

The tests were performed with a classic H-shaped microbial fuel cell (MFC). The MFC uses a bioanode through which the recirculation flow from the biogas reactor (UASB) passes and the biocathode is connected in parallel to the flow of the column photobioreactor (PBR). The experiments with integrated MFC in a laboratory installation (Fig. 1) were performed at a contact time of 10 days for the process of biomethanation in a continuous mode and in a periodic mode of cultivation of microalgae in a columnar photobioreactor.

UASB-bioreactor has a geometric volume of 6 dm³ and a working volume of 5 dm³ and is a cylindrical vessel with elliptical bottoms made of stainless steel with a ratio of diameter : height = 1 : 5. At three different heights in the cylindrical part of the reactor there is a possibility for taking liquid samples. The recirculation diaphragms pump (6) - model ISI 801, allows homogenization in pulse mode of the reactor volume at a maximum flow rate 8 dm³/h. In the bioreactor it is possible to maintain the temperature through an adjustable electric heater placed in the lower part of the reactor. In all experiments, the temperature was maintained in the range of 33°C - 35°C. The volume of gas emitted is read using a milli- gascouter “Ritter MGC-1” at a maximum flow rate of up to 1 dm³/h. The contents of CO₂, CH₄, O₂, H₂S and H₂ in the emitted gas were measured using a portable gas analyzer “Draeger X-am 7000”.

The column photobioreactor is a plexiglass tube with an inner diameter of 85 mm, a height of 630 mm and a working volume of 2.5 dm³. For efficient photosynthesis along the length of the photobioreactor is placed a plexiglass tube with an inner diameter of 36 mm in which is mounted a fluorescent lamp - type “SunGlo” with a power of 20W in lighting mode 12 h light : 12 h
dark. The culture fluid is recycled at flow rate 10 dm$^3$ h$^{-1}$ via peristaltic pump (6) through the cathode zone of the MFC (5) with a volume of 0.5 dm$^3$. From the bottom of the photobioreactor (PBR) is provided the possibility of aeration with air through an air pump. The laboratory installation provides the possibility for continuous (online) measurement of dissolved oxygen, pH, voltage, electrical conductivity, temperature and illumination, using Vernier® BTA sensors and visualization and recording of data via the LabQuest® interface.

The used microbial fuel cell is a classic H-shaped construction, with equal volumes of the anode and cathode chambers of 0.5 dm$^3$. Graphite rods with a diameter of 8 mm and a length of 100 mm were used for the electrodes. As a separator, a cation exchange membrane type - CMI-7000S (Membrane International Inc.) with an inner diameter of 30 mm was used. The studies of the operation of MFC were conducted in 2 modes - mode without external electrical load between the anode and cathode and mode when the fuel cell is loaded with an external resistance of 300 Ω. The two modes of operation were realized under identical conditions of the processes of biomethanation and photosynthesis during the 2 modes of MFC, each with duration of 60 days. A characteristic feature of the periodic operation of the photobioreactor was that it was recharged every 30 days with fresh nutrient medium and inoculum, i.e. twice during the experiment. The UASB biogas reactor in this experiment operated in a continuous mode with a contact time of 10 days, for the entire period of 60 days.

**Analytical methods**

Chemical oxygen demand (COD) and volatile fatty acids (VFA) were analyzed by standard methods APHA [14]. The pH was measured using pH electrode (VWR) and pH meter HANNA HI 9021. The redox potential (Eh) was measured using Sen Tix ORP sensor (WTW). The sulfate concentration was determined using spectrophotometric method at λ = 420 nm using BaCl$_2$ as reagent. The concentration of hydrogen sulfide in liquid phase was measured using a Nanocolor test 1-88/05.09 at λ of 620 nm.

**Wastewater**

The ethanol stillage used in the research was provided by “Kechlibar” LLC, village Svetovrachane, Bulgaria. Samples of wastewater were taken 3 times a year and stored in a refrigerator. 

**Microbial consortia for biomethanation process**

A microbial consortium from activated sludge of the WWTP - Sofia methane tanks was used. The bioreactor was fed with the substrate of ethanol stillage with a preliminary pH adjustment to 7.5 with 1N NaOH.

**Photosynthesis process and microalgae**

For photosynthesis process a columnar photobioreactor was constructed for the cultivation of microalgae isolated from natural habitats of Chlorella sp strain. The growth curve of the microalgae used in the photobioreactor on a modified nutrient medium - BG11 was traced [15] with the following composition for 1 L: 1.5 g NaNO$_3$, 0.5 g Na$_2$CO$_3$, 0.04 g K$_2$HPO$_4$, 0.075 g MgSO$_4$·7H$_2$O, 0.036 g CaCl$_2$·2H$_2$O, 0.045 g Citric acid, 0.0015 g Ferric ammonium citrate, 0.045 g EDTA (disodium salt), and 1 ml trace elements solution consisted of 2.86 g L$^{-1}$ H$_3$BO$_3$, 1.81 g L$^{-1}$ MnCl$_2$·4H$_2$O; 0.222 g L$^{-1}$ ZnSO$_4$·7H$_2$O; 0.39 g L$^{-1}$ NaMoO$_4$·2H$_2$O; 0.079 g L$^{-1}$ CuSO$_4$·5H$_2$O; 0.0494 g L$^{-1}$ Co(NO$_3$)$_2$·6H$_2$O. The amount of inoculum with microalgae was 10 % of the volume of photobioreactor and cathode zone of the MFC (total volume - 3.0 dm$^3$). The cultivation of microalgae was carried out at room temperature in the range 23°C - 25°C. PBR was aerated by air pump with a flow rate of 2.5 L h$^{-1}$ without addition of CO$_2$. Distilled water was added daily to compensate evaporation loss.

**RESULTS AND DISCUSSION**

For the process of photosynthesis, a column photobioreactor (PBR) was used for the cultivation of aerobic microalgae, connected in series with the cathode zone of the MFC (Fig. 1). To establish the growth curve of the microalgae used in the photobioreactor in parallel with the measured optical density and the number of microalgae was determined by a Burker counting chamber.

Cultivation of microalgae (batch) in PBR was continued for a period of 30 days and samples of the culture suspension periodically taken to determine cell number and optical density (OD). The obtained results (Fig. 2) show reaching the stationary phase in about 20 days, with the exponential (Log) phase lasting between 5 and 20 days from the start of cultivation. To determine the influence of the photosynthesis phases of oxygen microalgae on the cathode potential of a microbial fuel cell, the dynamics of the cathode half-cell potential in the cathode zone of the microbial fuel cell relative to
the standard potential of the calomel electrode was taken into account. The culture medium in the photobioreactor (PBR) after a 30-day period was replaced with fresh while retaining 1/10 of the old culture fluid in the reactor.

From the obtained time diagram (Fig. 3) of the cathode potential, pH and dissolved oxygen in the cathode zone of MFC the dynamics of the cathode potential depending on the concentration of dissolved oxygen during the dark and light phases of photosynthesis during the Log phase of the growth curve of microalgae is established. The established values from the time diagram show a change in the cathode potential (CP) in the range of 305 - 345 mV, pH in the range of 7.6 - 9.1 and dissolved oxygen from 7.6 to 8.4 mg L\(^{-1}\).

For the process of biomethanation, studies have been made with a typical waste substrate from the production of ethanol, namely an ethanol stillage. The ethanol stillage is a liquid substance that dropped after distillation of alcohol from the fermentation medium to produce ethanol from starch. It is characterized by a high

![Fig. 1. Scheme of the laboratory installation of MFC integrated with biomethanation and photosynthesis processes: 1 - feed substrate for biomethanation, 2 - UASB-biogas reactor, 3 -microbial fuel cell (MFC), 4 - column photobioreactor, 5 - recirculated flow through the anode zone of MFC and UASB-reactor, 6 and 7 recirculation pumps , 8 - load chain of MFC, 9 - biogas outlet, 10 - collector tank.]

![Fig. 2. Growth curve and change in optical density (at 650 nm) in columnar PBR.]

![Fig. 3. Time diagram of the dynamics of the cathode potential, pH and dissolved oxygen in the cathode zone of MFC during the dark and light phase of photosynthesis.]

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content of organic matter, which makes it a hazardous pollutant when disposed of directly in the environment. This organic waste is characterized by high values of COD - from 25000 mg L\(^{-1}\) to over 60000 mg L\(^{-1}\), has a variable chemical composition and very often in various processes of pretreatment of primary raw materials for hydrolysis of organic matter there is a relatively high sulphate content. The high concentrations of sulphates (in some cases above 1 g L\(^{-1}\)) are due to the practical treatment of the primary raw material with a solution of H\(_2\)SO\(_4\), in order to hydrolyze the organic substances. The bioreactor was fed with the ethanol stillage substrate, with a preliminary pH adjustment to 7.5 with 1N NaOH and a peristaltic pump flow rate of 0.5 dm\(^3\) for 24 h, which corresponds to a hydraulic residence time (HRT) of 10 days.

The results obtained from the main technological parameters (Table 1) show a high degree of biodegradation of the organic substrate to COD - from 93.3 % to 68.8 %. The presence of sulfates in the ethanol stillage is the main reason for the presence of HS\(^-\) in the stock solutions. The latter is also confirmed by the measured gas composition of the separated biogas: CH\(_4\) 55.3 - 69.2 %, CO\(_2\) 27.2 - 34.6 %, H\(_2\)S 0.5 - > 2.0 %, H\(_2\) 267 - 1587 ppm and O\(_2\) 0.3 - 1.2 %. The amount of biogas released after the 30th day, when the process stabilizes, varies from 2.240 dm\(^3\) to 2.850 dm\(^3\) for 24h.

From the obtained results it is obvious that a concomitant process of microbial sulfate reduction (MSR) is taking place. In many cases, microbial sulfate reduction is also a more energy-efficient process than methanogenesis [16], in a case of the electron donor in the system (acetate and H\(_2\)). Intensive MSR also creates a number of difficulties in biogas production - increased hydrogen sulfide content in biogas, the need for purification of biogas, corrosion of equipment and harmful effects on the environment and the working environment.

The integration of MFC in the system (Fig. 1) was done after the 30th day of charging and inoculation of the UASB bioreactor to the anode zone and on the 5th day of culturing the aerobic microalgae in PBR to the cathode zone. In order to establish the influence of the fuel element on the process of biomethanogenesis, the work of MFC was realized in two variants - mode without external electrical load between the anode and cathode, and mode with placement of external electrical load of 300 \(\Omega\), as in both options the main technological parameters are taken into account.

The obtained results from the power and polarization curves of the integrated MFC show a significant influence of the maximum values of power and current density from the conditions in the biocathode zone, as the highest established values of power and amperage are - 27.4 mW m\(^{-2}\) and 68 mA m\(^{-2}\), for 15 days from the cultivation of oxygen microalgae (Fig. 4 and Fig. 5) in the mode without external electrical load and 16.2 mW m\(^{-2}\) and 67 mA m\(^{-2}\) in the mode with electrical load. The results of the polarization curves (Fig. 4 and Fig. 5) are similar, where the highest values of the open circuit voltage (OCV) are also obtained for 15 days, respectively - 720 mV (without load) and 491 mV (with load). Interesting results were also obtained regarding the change in the gas composition of biogas with and without the inclusion of MGC in the load regime in the system (Table 2).

Table 2 shows the range of change of the gas composition in the two modes of MFC operation. It can be seen that a significant change in the content of CH\(_4\) (an increase of about 1 %) and CO\(_2\) (a decrease of 1 - 2 %) is not observed, but in terms of the content of H\(_2\)S, it is

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Input</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.5 ± 0.1</td>
<td>6.8 - 7.7</td>
</tr>
<tr>
<td>COD, mg O(_2) L(^{-1})</td>
<td>33500 - 27428</td>
<td>2215 - 8562</td>
</tr>
<tr>
<td>VFA, mg L(^{-1})</td>
<td>1256 - 1034</td>
<td>754 - 346</td>
</tr>
<tr>
<td>SO(_4), mg L(^{-1})</td>
<td>950 - 1141</td>
<td>25 - 50</td>
</tr>
<tr>
<td>ORP, mV</td>
<td>65 - 76</td>
<td>-355 - -376</td>
</tr>
<tr>
<td>HS(^-), mg L(^{-1})</td>
<td>-</td>
<td>33.5 - 47.2</td>
</tr>
</tbody>
</table>
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not - in load mode, $H_2S$, practically it is not established, while in the mode without loading of MFC in some cases the content increases to over 2 %, i.e. successful removal of $H_2S$ is performed by integrating MFC into the biogas reactor. This result shows that the hydrogen sulfide produced in the medium during the parallel process of microbial sulfate reduction (in the form of $HS^-$ ion) is successfully oxidized on the anode surface in MFC mainly to $S^0$ and other forms of sulfur, thus acting as a mediator in electronic transmission [10]. The established content of sulfates, COD and $HS^-$ in the outgoing liquid phase of the biogas reactor (Table 3) is a further confirmation of the ongoing processes of sulfate reduction and oxidation of $H_2S$ in the anode zone of the fuel cell.

According to the results presented in Table 3, in the fuel cell load mode, $HS^-$ is absent in the middle and the COD value decreases from 17 % to 20 %. This is obviously due to the oxidation processes in the bioanode area.

Previous studies have found the dependence of OCV (open circuit voltage) on MFC on the concentration of

**Table 2. Biogas composition with integrated microbial fuel cell.**

<table>
<thead>
<tr>
<th>Component</th>
<th>Without external electrical load</th>
<th>With external electrical load (300 Ω)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CH_4$, %</td>
<td>55.2 - 68.2</td>
<td>56.3 - 69.1</td>
</tr>
<tr>
<td>$CO_2$, %</td>
<td>27.2 - 33.3</td>
<td>26.3 - 31.4</td>
</tr>
<tr>
<td>$H_2S$, %</td>
<td>0.5 - &gt;2.0</td>
<td>0.01 - 0.1</td>
</tr>
<tr>
<td>$H_2$, ppm</td>
<td>271 - 1585</td>
<td>147 - 1249</td>
</tr>
<tr>
<td>$O_2$, %</td>
<td>0.2 - 1.1</td>
<td>0.3 - 1.2</td>
</tr>
</tbody>
</table>

**Table 3. Values of the main technological parameters in the liquid phase of the UASB bioreactor output at different operating modes of the integrated MFC.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MFC without electric load</th>
<th>MFC with electric load (300 Ω)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.8 - 7.7</td>
<td>6.5 - 7.3</td>
</tr>
<tr>
<td>COD, mg $O_2$ L$^{-1}$</td>
<td>3492 - 4378</td>
<td>2892 - 3474</td>
</tr>
<tr>
<td>VFA, mg L$^{-1}$</td>
<td>754 - 346</td>
<td>541 - 214</td>
</tr>
<tr>
<td>$SO_4$, mg L$^{-1}$</td>
<td>45 - 65</td>
<td>105 - 55</td>
</tr>
<tr>
<td>ORP, mV</td>
<td>-355 - -385</td>
<td>-345 - -364</td>
</tr>
<tr>
<td>$HS^-$, mg L$^{-1}$</td>
<td>47.2 - 95.2</td>
<td>-</td>
</tr>
</tbody>
</table>
hydrogen sulfide in the medium [17]. This can also explain the lower values of OCV in the load mode (Fig. 4), where hydrogen sulfide is not detected in the liquid phase (Table 3).

CONCLUSIONS
The integration of MFC between biomethanation process and aerobic photosynthesis has been realized. The values of the main technological parameters in the zones of the bioanode and biocathode have been established. Positive effects on the biomethanation process are: the removal of hydrogen sulfide from biogas and a higher degree of mineralization of the organic substrate. The obtained results are basis for future research in order to upgrade the biomethanation process through the use of MFC.

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