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# Efficiency of algae-assisted photo-bioelectrochemical system in anaerobic wastewater treatment

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Bioelectrochemical systems offer various potential applications for environmental protection. The present study examines the performance of a photo-bioelectrochemical system (PBES) consisting of a microbial fuel cell (MFC) and a column photobioreactor with a mixed culture of microalgae. The generation of significant amounts of oxygen in the medium by the algae during photosynthesis is a key factor in their usage in the cathodic zone of bioelectrochemical systems. In the anodic area of a microbial fuel cell, two waste organic substrates (ethanol stillage and vinasse) used as electron donors for the microbial sulfate reduction process were compared. At different operating modes, there were determined and analyzed the main electrochemical parameters of a PBES - open circuit voltage (436 – 510 mV), maximum power (8.0 – 14.5 W/m$^2$), current density (34.8- 64.1 mA/m$^2$), internal resistance, coulombic efficiency, etc.

**Keywords:** Bioelectrochemical systems, Coulombic efficiency, microalgae, microbial fuel cells, oxygen generation, microbial sulfate reduction.

## INTRODUCTION

The current economic situation and the required sustainable development in terms of the environment create an urgent need to develop energy-efficient and eco-friendly technologies for the treatment of wastewater fluids [1]. Innovative technologies in the field of bioelectrochemical systems (BES), such as microbial fuel cells (MFCs) and MFCs with microalgae (mMFC) cultivated in photobioreactors (PBR), create the opportunity of electricity generation, $CO_2$ removal from gas mixtures and wastewater treatment [2].

MFCs can successfully be applied to remove both organic and inorganic pollutants from wastewaters [3]. The main process is the oxidation of an organic electron donor, and the removed electrons are transferred to an insoluble anode of the BES, instead of the corresponding natural acceptors (oxygen, sulfates, ferric ions, nitrates, etc.) [4]. The typical MFC consists of anode and cathode chambers separated by an ion exchange membrane. BES such as MFCs can also be used for microalgae cultivation and bioelectricity production, offering advantages over conventional microalgae cultivation systems.

Microalgae microbial fuel cells are a type of MFCs with microalgae as a biocatalyst. The photobioreactor can function as part of the cathode zone of the mMFC for the cultivation of microalgae [5]. Oxygen generation in the cathode zone is achieved by a connected PBR or by cultivating microalgae directly in the cathode zone [6]. Photobioreactors are closed systems (isolated from the environment) for cultivating microalgae in transparent containers with different shapes and sizes. These systems have a number of advantages, such as low contamination levels, high productivity, easy control of temperature and pressure, good light illumination, small water losses [7]. The most commonly used types of PBRs are tubular (cylindrical), column type with aeration, flat, combinations of several types, etc.

Combined BES with microalgae in the cathode zone and anaerobic processes (biomethanization, microbial sulfate reduction, denitrification, etc.) in the anode zone are the preferred option because the cultivation of microalgae in the cathode zone (mMFCs) increases the oxygen content as a result of photosynthesis, which increases the electricity production. In addition, the process reduces the amount of $CO_2$ [8].

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Recent studies have shown that various types of wastewater can be used as an organic substrate in the anode zone, which increases the efficiency of the system [9]. Typical examples of such waste organic substrates are ethanol stillage and vinasse. The ethanol stillage is a liquid waste from the distillation of ethanol from alcoholic fermentation of raw materials containing starch (corn, wheat, barley, etc.). The vinasse is derived mainly from the fermentation and distillation of molasses, most often in the production of ethanol from sugar cane or sugar beet. Organic wastewaters from beer production, such as ethanol stillage and vinasse, are substrates that are successfully utilized in the anaerobic biomethanization process. They are characterized by high acidity (pH: 3.5-4), high organic content (COD: 50 - 150 g/l), and often contain large amounts of nitrogen compounds and sulfates [10].

It is important to pay attention to the high concentration of sulfates in these waste effluents. High sulfate concentrations can affect further water treatment. During anaerobic digestion, sulfates are converted to more toxic sulfides (e.g. hydrogen sulfide). In general, inhibition of the biomethanization process by sulfides does not occur when the COD/$SO_4$ ratio in the wastewater is greater than 10 g/g. Inhibition of anaerobic digestion is strong when the COD/$SO_4$ ratio is less than 0.5 g/g. Thus, vinasse and ethanol stillage can form wastewaters that are difficult to treat, not only due to their characteristics, but also due to their significant volume [11].

Sulfate removal in nature is achieved through the process of microbial sulfate reduction (MSR). This process is widely used in bioelectrochemical systems such as MFCs [12]. In heterotrophic MSR, the process takes place in the anode zone of the MFC, and it is known that in this case there is no necessity of additional mediators, since the hydrogen sulfide plays the role of such, and the generation of electricity in the system occurs during the oxidation of part of the produced hydrogen sulfide on the surface of the anode [13, 14]. An important effect of the process is the reduction of sulfates to biogenic $H_2S$, which is a mediator in the electron transfer, being oxidized in the anode chamber on the anode surface to elemental sulfur ($S^0$) and its other forms [12]. Unlike classical MFCs, where during the oxidation of an organic electron donor, the electrons taken away are transferred to the insoluble anode, in this case, a significant part of the electrons from the organic substrate go to the reduction of sulfates. Sulfate-reducing bacteria (SRB) and electrogenic microorganisms often use the same electron donors, such as lactate, acetate or propionate. In the anode chamber of the MFC, SRB can reduce sulfate to sulfide using these donors, which can reduce their availability to electrogenic bacteria and thus limit electricity production. Furthermore, in terms of electron transfer mechanisms, SRB can participate in direct or indirect extracellular electron transfer (EET) to the anode. These mechanisms include direct contact, use of conductive nanowires, or redox mediators [12].

The main objective of this study is to analyze the performance of a newly designed photo-bioelectrochemical system combining a photobioreactor and an MFC. Another important task is to determine the efficiency of mMFCs with vinasse and ethanol stillage as electron donors for the MSR process in the anode zone of the fuel cell, and utilizing them, removing the sulfates and producing energy.

## MATERIALS AND METHODS

### *Substrates, inoculum and enrichment of microbial communities*

For the microbial sulfate reduction process in the anode zone of mMFC were used two types of wastewater - vinasse (from wine brandy production) and ethanol stillage (from a plant for ethanol production from hydrolyzed wheat using a sulfuric acid solution). The wastewaters used in this study are characterized in Table 1.

**Table 1.** Basic characteristics of the wastewater

| Parameter | Vinasse | Ethanol stillage |
|---|---|---|
| pH | 3.41 | 3.52 |
| TKN (Total Kjeldahl nitrogen), mg/l | 159.3 | 103.7 |
| COD, $gO_2$/l | 53.6 | 79,2 |
| Dry matter, % | 3.11 | 3.31 |
| $SO_4^{2-}$, g/l | 0.969 | 1.165 |

Before usage for the MSR process in the anode zone of mMFC, the vinasse and ethanol stillage were diluted with distilled water in a ratio of 1:1, the pH was adjusted to 7.5 using 4N NaOH solution and anhydrous $NaSO_4$ was added until the sulfate concentration reached 3.2 g/l. During the experiments, the solutions with organic substrates were stored at 4 °C in a refrigerator.

A mixed culture of microalgae of *Scenedesmus* sp. and *Chlorella* sp., isolated from natural water sources, was used in the cathode zone of the mMFC, which were cultivated on a modified nutrient medium BG11, with a chemical composition specified in a previous study [15]. The amount of microalgae inoculum was approximately 10% of the working volume of the photobioreactor and the

cathode zone of the MFC. The cultivation of microalgae was done at room temperature in the range of 22 - 25 ºC.

For the process of heterotrophic microbial sulfate reduction, a microbial consortium isolated from natural habitats and immobilized on natural zeolite - clinoptilolite was used.

The pretreatment and characterization of the natural zeolite used have been presented in a previous study [13]. The composition of the microbial consortium of sulfate-reducing bacteria and metabolically related groups of microorganisms has been presented in a previous study [14].

*Laboratory set up description*

A new design of a combined photo-bioelectrochemical system was used for the experiments, including a microbial fuel cell (MFC) and a column-type photobioreactor integrated into the cathode zone (Figs. 1 and 2).

The base of the bioelectrochemical system is 3D printed from PETG (glycol-modified polyethylene terephthalate) filament, containing the anode (with a volume of 0.1 $dm^3$) and cathode zones (with a volume of 1 $dm^3$).

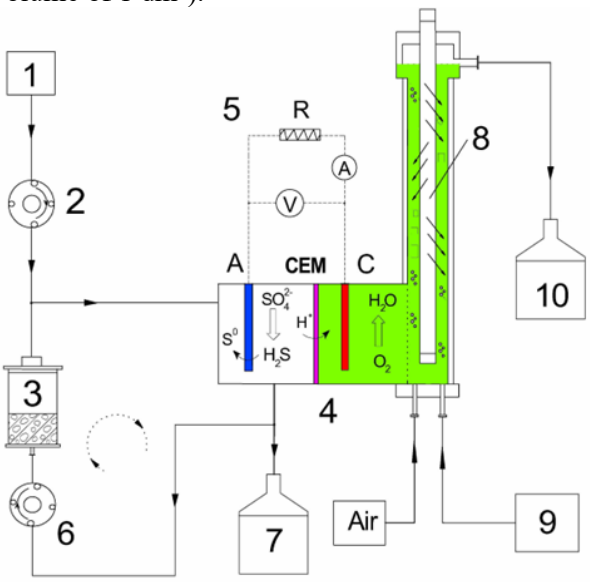


**Figure 1.** Experimental setup. 1 - organic substrate input, 2 - dosing peristaltic pump, 3 - sulfidogenic bioreactor, 4 - mMFC, 5 - load circuit between anode and cathode, 6 - recirculation pump, 7 - output solution after MSR, 8 - photobioreactor, 9 - nutrient medium for microalgae, 10 - output solution from PBR.

For the cathode and anode, two identical graphite plates with dimensions 100x100x6 mm were used. For the separator in mMFC, a cation exchange membrane type CMI-7000 with dimensions 100x100 mm was used. The column-type photobioreactor (made of plexiglass) is connected to the cathode zone of the MFC, together forming a working volume of 3.5 $dm^3$. In the central part of the photobioreactor a tubular LED light source (8) is placed with a power of 16 W and a wavelength in the range of 400÷700 nm, which provides a light flux with an intensity in the range of - 3600 - 4500 Lx, in mode – 12 h light to 12 h dark.

The oxygenic photosynthesis zone in the combined photobioreactor with the cathode zone of the mMFC (3) is supplied with air at a flow rate of 1.5 $dm^3$/60 s, without additional $CO_2$ (Fig. 1).

For the cultivation of SRB in the anode zone of mMFC, a sulfidogenic bioreactor with immobilized biomass was used (position 3 in Fig. 1), in which approximately half the volume of 0.7 $dm^3$ was filled with 0.3 kg of modified zeolite.

The described mMFC design consists of two zones - an anaerobic anode zone, where the electroactive biofilm of SRB extracts electrons from waste organic substrates, reduces sulfates to $H_2S$, which in turn is oxidized on the anode to $S^0$ and an aerobic cathode zone, where the oxygen produced during the light phase of photosynthesis is the final electron acceptor and reacts with the released protons passed through the CEM, resulting in water (Fig.1).

**Figure 2.** The mMFC and the laboratory setup.

*Operations of laboratory installation*

Maintaining constant high values of the cathode potential is directly dependent on the amount of oxygen produced in the catholyte by the oxygenic microalgae [16]. For this purpose, in advance, in a periodic cultivation mode, the stages of microalgae growth, the change in cathodic and anodic potentials (relative to a comparative calomel electrode) and the oxygen concentration in the catholyte were determined. Regarding the anode zone of the mMFC, where the MSR process is implemented, in previous studies [17], optimal contact times in the range of 14 to 34 h have been determined for the two types of wastewater used. The continuous supply of the anode zone with waste organic substrate (vinasse and/or ethanol stillage) was realized by peristaltic pump 2 (Fig. 1), with a flow rate of 300 $dm^3$/24 h, in a working volume of 400 $dm^3$ (100 $dm^3$ - volume of the anode zone in the mMFC and 300 $dm^3$ - volume of the liquid phase in the sulfidogenic bioreactor), achieving a contact time of 30 h. During the experiments, the anolyte was continuously recirculated between the volume of the sulfidogenic bioreactor and the anode zone of the mMFC, by peristaltic pump 6 (Fig. 1).

### *Analytical methods*

At various points in the laboratory installation is provided the possibility of sampling and continuous (online) measurement of dissolved oxygen, pH, voltage, electrical conductivity, temperature and light illumination, using Vernier$^R$ BTA sensors and visualization and recording of data *via* the interface LabQuest$^R$.

Chemical oxygen demand (COD) was measured with Merck instruments reagents according to APHA [18]. pH and ORP were measured with pH/ORP/EC-Meter "Elmetron CRC-461". The sulfate concentration was determined using a spectrophotometric method at λ - 420 nm, using a $BaCl_2$ reagent. The concentration of hydrogen sulfide in the liquid phase was measured using Nanocolor test 1-88/05.09 at λ = 620 nm.

A light microscope (Boeco$^R$, BM-800) was used to observe the microalgae and their growth phases; the optical density of the cell suspension was determined during the cultivation of the microalgae at a wavelength of 650 nm and a red filter.

### *Electrochemical analysis*

The electrical parameters of mMFC were measured with a digital multimeter Mastech MS8229, and a precision potentiometer with a range of values from 10 Ω to 11 kΩ was used for the load resistance. In this range of external resistance variation, the polarization characteristics and power curves were also measured. A fixed external load resistance value of 100 Ω was used during the experiments, which was optimal in the mMFC power curves shown below.

The power density (P), relative to the geometric anode surface area, was calculated using the equation $P=U^2/(R_T.A)$, where $A(m^2)$ is the anode surface area, RT (Ω) is the external load resistance, and U (V) is the mMFC voltage.

To measure the cyclic VA-characteristics of mMFC, a potentiometer Squidstat Plus, with a calomel reference electrode, was used at a scan rate of 1 mV/s, in the range from 750 mV to -750 mV, and the data were visualized and recorded in real time on a computer.

The value of the coulombic efficiency (CE) was determined by the value of COD in the anolyte as the difference between the values without external resistance ($R_T$) and with resistance. In this way, a distinction can be made as to what part of the organic substances in microbial sulfate reduction are oxidized biologically and what part electrochemically to produce electricity.

For the calculation of CE was used:

$$CE = \frac{M.I.t}{F.b.V_{an}.\Delta COD.} x\,100\,\% \quad (1)$$

where: M= 32 - molar mass of $O_2$, t(s) - time, I - average current value during the experiment (A), F (Faraday constant) = 96845 C/mol, b = 4 – number of electrons required for oxidation of 1 mol $O_2$, ΔCOD – difference between the initial and final COD values ($gO_2$/l), $V_{an}$ – volume of the anode chamber (l).

## RESULTS AND DISCUSSION

### *Influence on the cathodic and anodic potential of mMFC in batch mode*

Initially, to determine the optimal values of the cathode potential of the mMFC, the microalgae were cultivated in batch mode for 22 days, during which the anode and cathode potentials, as well as the dissolved oxygen and optical density in the catholyte, were measured. In the same time, in the anode zone, an MSR process was started, with ethanol stillage as electron donor. Figure 3 shows the change in the values of the cathodic and anodic potential, the oxygen concentration, and the dynamics of the optical density of the catholyte. The results show that the highest values of the cathodic potential are obtained at the end of the exponential phase (between 10 and 15 days), which is consistent with the results of a previous study [15]. It was found that after 5 days, the anodic potential stabilized in the

range of 215 - 226 mV. These potential values and oxygen concentrations were measured during the light phase of photosynthesis.

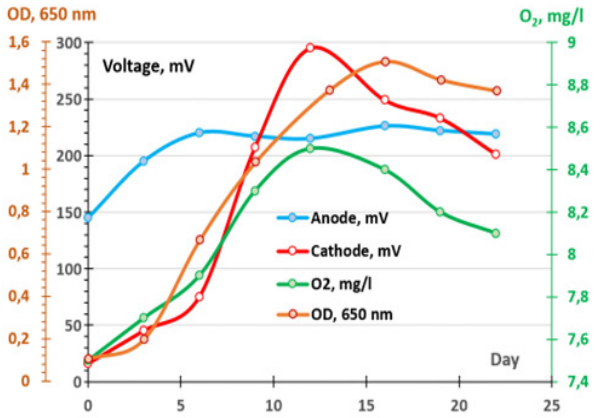


**Figure 3.** Dynamics of anodic potential, cathodic potential, dissolved oxygen and optical density (OD).

Based on these results, the PBR (resp. the cathode zone of the mMFC) was fed daily with fresh nutrient medium (modified BG11) with a flow rate of 350 $dm^3$/24h, achieving a contact time of 10 days (Fig. 1), which in this case was considered optimal with respect to the cathode potential of the mMFC.

*Research on the influence of the electron donor on the electrochemical characteristics of the mMFC*

To study the influence of ethanol stillage and vinasse (electron donors in the anode zone of mMFC) on the efficiency of the mMFC, polarization curves and power curves were plotted for both substrates (Fig. 4). These curves were measured during the light phase of photosynthesis, in continuous operation mode, in both anode and cathode zones. From the data above, it is evident that the maximum power density for ethanol stillage is 14.5 W/$m^2$, and for vinasse it is 8.0 W/$m^2$. The current density values are respectively 64.1 mA/$m^2$ (ethanol stillage) and 34.8 mA/$m^2$ (vinasse).

These values were obtained at a fixed external load resistance of 100 Ω (for the ethanol stillage) and 200 Ω (for the vinasse), which shows that the corresponding internal resistances of the mMFC are close to these values. A more accurate analysis of the internal resistance ($R_{int}$) was made based on the cyclic voltammetry characteristic presented in Fig. 5. Accordingly, for two linear sections of these characteristics, it was found that the internal resistance of the ethanol stillage is 83.3 Ω, and that of the vinasse is 125 Ω. The lower internal resistance of the ethanol stillage variant compared to the vinasse is probably due to the significant difference in their electrical conductivities (Table 2), both in the initial substrate and in the anolyte with and without load. When analyzing the cyclic voltammetry characteristics, additional data were obtained for the two studied variants. Accordingly, the slope of the cyclic voltammetry characteristic curve for the ethanol stillage is greater than that for the vinasse, and significant differences in their areas are also noticeable (Fig. 5).

Determining the coulombic efficiency (CE) in a microbial fuel cell with an MSR process in the anode zone is an interesting problem. CE in a microbial fuel cell is calculated as the ratio between the actual electrical charge produced and the theoretical electrical charge that could be obtained from the complete oxidation of the supplied electron donor. Measurements of the COD change with and without mMFC load resistance were made to separate what part of the electron donor is spent on the MSR process and what part on electrogenesis, and are presented in Tables 2 and 3.

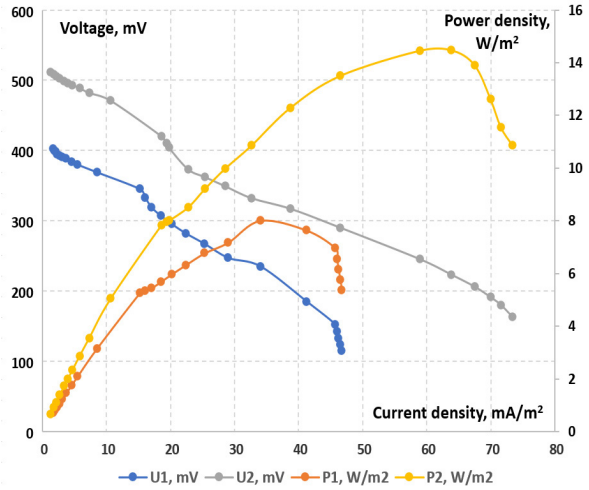


**Figure 4.** Polarization curves and power curves of mMFC with different electron donors in the anode zone. U1, P1- voltage and power density in ethanol stillage, U2, P2- voltage and power density in vinasse.

**Table 2.** Values of the main technological parameters in the anolyte, with and without mMFC load.

| Parameter | $SO_4$, mg/l | EC, mS/cm | COD, $gO_2$/l | $H_2S$, mg/l | OCV, mV |
|---|---|---|---|---|---|
| Ethanol stillage | | | | | |
| Input | 3234 | 15.12 | 39.6 | - | - |
| Output $R_T = \infty$ | 260 | 19.87 | 29.6 | 141 | 512 |
| Output $R_T$ = 100 Ω | 484 | 16.55 | 23.3 | 35 | 436 |
| Vinasse | | | | | |
| Input | 3320 | 13.08 | 28.6 | - | - |
| Output $R_T = \infty$ | 1095 | 18.34 | 24.2 | 118 | 350 |
| Output $R_T$ = 100 Ω | 1358 | 15.21 | 21.5 | 94 | 310 |

The results in Table 3 show that the rate of microbial sulfate reduction in the ethanol stillage is 99.1 mg/l.h (without load) and 91.7 mg/l.h (with load) , while in the vinasse it is 74.2 mg/l.h (without load) and 65.4 mg/l.h (with load). Accordingly, the degree of sulfate removal is 91.9% (ethanol stillage) and 67.02% (vinasse).

A relationship is observed between OCV (open circuit voltage) and the concentration of $H_2S$ in the anolyte, their values being higher in the stillage than in the vinasse.

The increase of the sulfates in the anolyte with the application of external load resistance (Table 2) is probably due to the stimulation of oxidation processes in the anode zone during the oxidation of $H_2S$. A particular interest from the obtained results is what part of the electron donor (ethanol stillage and vinasse) was oxidized as a result of microbial sulfate reduction (MSR), and what part is for electrogenesis when applying an electrical load ($R_T$), between the anode and the cathode of the microbial fuel cell (mMFC). The results (Table 3) show that the sulfates and the anode are in constant competition as electron acceptors for both studied substrates, and the sulfates are the preferred electron acceptor.

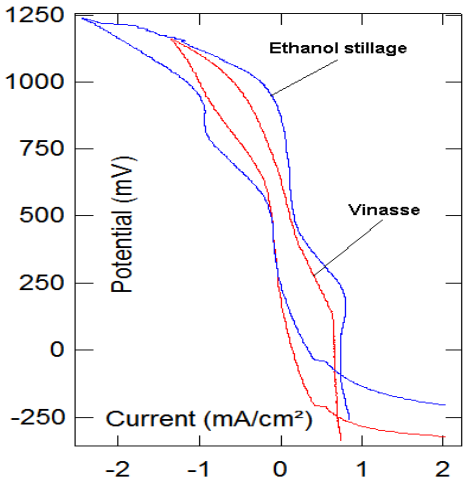


**Figure 5.** Cyclic voltammetry characteristics in mMFC with Ethanol stillage and Vinasse.

Fig. 6 shows the amount of substrate utilized, estimated by COD, for the MSR process (1), for electrogenesis (2) and that part of COD (3) that remains unused in these processes.

The ethanol stillage, compared to the vinasse, shows better results in terms of microbial sulfate reduction rate, COD and sulfate removal rate and lower internal resistance in the anode zone of mMFC. Regarding the obtained calculated values of the coulombic efficiency, similar values are found for ethanol stillage (17.1%) and vinasse (14.8%). These values for the coulombic efficiency are comparable to those obtained by Akgul *et al*. [19].

For both substrates studied, a higher relative participation in the MSR process was observed compared to electrogenesis – wth the ethanol stillage (25.5% *vs*. 15.15%), and with the vinasse (15.38% *vs*. 9.45%). This indicates that sulfate-reducing microorganisms are metabolically more active in the anodic zone and largely compete with electrogenic bacteria for available electrons. The better digestibility of organic substances when used for ethanol stillage compared to vinasse indicates the better bioavailability and biodegradability of organic compounds in the ethanol stillage.

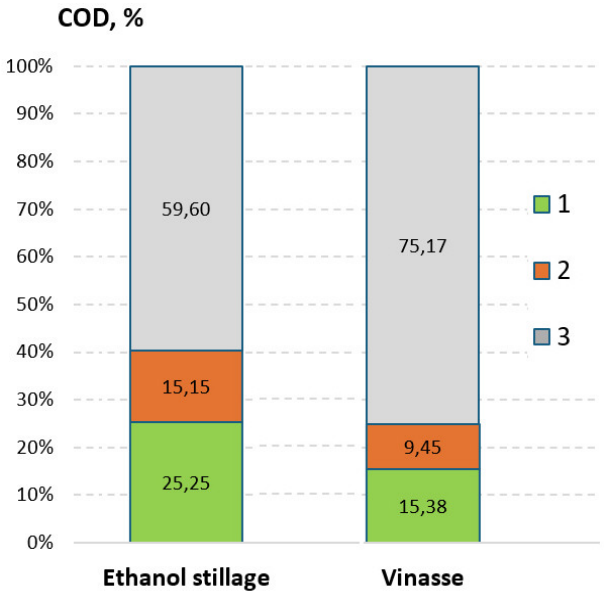


**Figure 6.** Comparison between the rates of COD absorption in the MSR process in the anode zone, with and without external load. 1 - only for the MSR process ($R_T$=∞), 2 - only for electrogenesis ($R_T$=100 Ω), 3 - residual part of COD.

**Table 3.** $SO_4$ and COD removal in the anolyte and coulombic efficiency (CE) values in mMFC.

| Anolyte | $SO_4$-reduction rate, $SO_4$, mg/l. h | $SO_4$-removal, % | COD-total removal, % | COD (without $R_T$), % | COD ($R_T$=100 Ω), % | CE, % |
|---|---|---|---|---|---|---|
| Ethanol stillage | 99.1*-91.7** | 91.9*- 85.0** | 41.2 | 25.3 | 15.9 | 17.1 |
| Vinasse | 74.2*- 65.4** | 67.0*- 59.1** | 24.8 | 15.4 | 9.4 | 14.8 |

*- with open circuit, **- with external load ($R_T$=100 Ω)

## CONCLUSIONS

A combined bioelectrochemical system of a photobioreactor and MFC was studied. The efficiency of the combined mMFC depends on both the cathodic and anodic potentials, which are influenced by various medium factors, and on the type of electron donor for the MSR process in the anode zone. The competition between microbial sulfate reduction (MSR) and electrogenesis in the anode chamber of the mMFC, especially with respect to the distribution of electron donors between these two processes, is decisive for the operating efficiency of the studied photo-bioelectrochemical system.

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