Original Research Article

Identification and Characterization of Natural Habitats of Electrochemically Active Bacteria

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ABSTRACT

Aims: This study aims to define criteria for the main physical and chemical characteristics of the environmental niches populated with electrochemically active microorganisms, capable to perform anaerobic respiration and potentially used in bio-electrochemical systems such as Microbial Fuel Cells.

Study design: In this study, specific parameters of the environment in waterbodies (such as lakes, streams etc.) and their bottom layers are analyzed. The main parameters of interest include the concentration of dissolved oxygen in the water column, the organic matter content in the sediments and the presence of alternative electron acceptors (such as iron and manganese ions) to support anaerobic respiration. Sediment microorganisms are characterized for their electrochemical and biodegradation activity.

Place and Duration of Study: The tested sediment and water samples were collected from "Poda" Protected Site located on the outfall of Lake "Uzungeren", south of City of Burgas, BULGARIA

Methodology: The samples were analyzed employing TGA, ICP and microbiological methods focusing on chemical, physical and biological conditions available for anaerobic respiration in this ecological niche.

Results: The results show very low concentrations of dissolved oxygen (from 1.4 to 2.2 mg/dm^3 in the various locations). The conductivity and the pH values measured were relatively high and the mean values obtained are 5230 µS/cm and 8.2 respectively. The sediment samples demonstrated very high organic matter content (22.5% of the dry mass) and relatively high levels of iron and manganese.

Microbial fuel cell powered by mixed bacterial culture isolated form the tested sediment samples demonstrated stable performance reaching power density of 3.5 W/m^2 and the COD removal rate of 42 mgO_2/dm^3 per day

Conclusion: The results confirms the initial hypothesis that electrochemically active microorganisms are available in environments with high concentration of organic matter, iron and manganese in combination with low availability of dissolved oxygen. Mixed culture of anaerobic bacteria isolated from the tested sediment sample was successfully implemented to power Microbial Fuel Cell

Keywords: microbial fuel cell, anoxic respiration, lake sediments

1. INTRODUCTION

Since in the past years, anaerobic digestion has been employed as a main biotechnological approach to recover energy from the waste organic matter, and that occurs by converting the complex organic compounds into methane containing biogas [1]. In a second stage, methane needs to be enriched and then converted into electricity by combustion in cogeneration systems. Recently, an alternative for direct recovery of waste streams into electricity by Bio-electrochemical processes was demonstrated in many studies and the Microbial fuel cells (MFC) are the most popular example in this regard [2]. In this type of reactors, simultaneously with the wastewater treatment, organic pollutants equivalent to 1kg of COD could be converted to up to 4 kWh electrical energy [3]. This is possible due to the presence of the so-called electrogenes, i.e. bacterial species that usually form electrochemically active biofilm on the MFC electrode surface [4].

Form the biochemical point of view, there is fundamental difference between the conventional anaerobic digestion and bio-electrochemical processes even though both processes are anaerobic. Anaerobic digestion is predominantly fermentative transformation of the substrates without involvement of any complex electron transport mechanisms for ATP synthesis. On the contrary, in the microbial fuel cell, a respiratory type of metabolism is implemented with all the typical energy benefits for the bacterial cell.

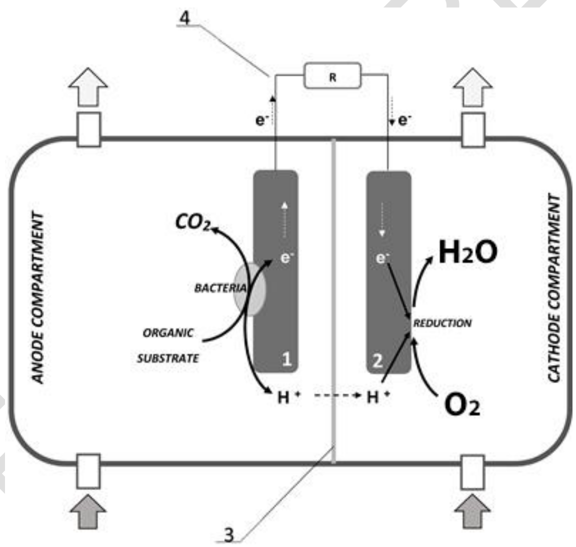

Figure 1. Experimental design and construction of the microbial fuel cell used in the experiments: 1 – anode; 2 – cathode; 3 – proton exchange membrane; 4 – external electric circuit.

Anaerobic cellular respiration is similar to aerobic cellular respiration in that electrons extracted from a fuel molecule (organic substrate or the pollutants in the treated wastewater) are passed through an electron transport system, driving ATP synthesis. The only difference between the two processes is the terminal electron acceptor due to the absence of oxygen attributed to the anaerobic conditions. Anaerobic respiration is a part of the biochemical mechanisms behind the global nitrogen, iron, sulfur, and carbon. These processes occur in variety of environments, including freshwater and marine sediments, soil, subsurface aquifers, deep subsurface environments, and biofilms [5].

In the MFC reactors, the organic substrates presented in the anode compartment are biologically oxidized in absence of oxygen. Under these conditions, the anode become terminal electron acceptor due to its high electrode potential. The electrochemically active microorganisms which drive the process are able to transfer the electrons obtained from the decomposition of the organic molecules directly to the anode surface by involving specific transfer mechanisms (direct “surface to surface” transfer or chemically mediated electron transport) [6,7]. The electrons then flow through an external electric circuit to the cathode where they are consumed in variety of cathode reactions such as reduction of metal ions or oxygen (Fig 1).

The implementation of a technology afore-mentioned is strongly dependent upon specific metabolic activity projected by electrochemically active biofilms. Isolation and characterization of the electrogenic bacteria is a crucial step toward the improvement and commercialization of the Bio-electrochemical processes such as MFC. In this sense, this study aims at defining the criteria for the main physical and chemical characteristics of the environmental niches populated with electrogenes, which can improve the future identification of potential habitats and sampling sources of microorganisms with this specific activity.

2. MATERIAL AND METHODS

2.1 GENERAL ANALYTICAL METHODS

The samples used in this study were collected from the bottom of "Poda" Protected Site located on the outfall of Lake “Uzungeren”, south of City of Burgas, BULGARIA (42°26'42.2"N 27°27'56.0"E). The tested specimens consist of the solid fraction and the filtrate obtained from the samples shortly named “sediment” and “water”.

The temperature, pH, dissolved oxygen and conductivity of the water were measured *in situ* with Hach HQ30D Portable Multi Meter.

The chemical oxygen demand (COD) was measured using HACH Lange cuvette tests (Product ID: LCK 314) and HACH Lange DR 3900 spectrophotometer.

2.2 THERMAL GRAVIMETRIC ANALYSIS (TGA)

NETZSCH STA 449 F3 TGA-DSC analyzer was used for the determination of water and total organics content of the sediment samples. The procedure was conducted under the following conditions: Temperature range of 25-600 ˚C, heating rate 15 °C/min and α-Al_2O_3 as reference. The mass loss of was plotted as a function of temperature.

2.3 PLASMA ATOMIC EMISSION SPECTROSCOPY (ICP-OES)

The sediment was dried in a vacuum oven (Salvis Rotkreuz, Switzerland), for 24h at 40°C/105°C. In the next step a precise weighing scale XS 205 DualRange (Mettler-Toledo GmbH (Giessen, Germany) was used to measure the weight of the dried samples. Afterwards the dried samples were pulverized with a ball mill MM 301, Retch (Haan, Germany).

The dried and pulverized samples were dissolved by a treatment with aqua regia, which involved 1 ml of HNO_3 and 3 ml of HCl, purchased from Sigma-Aldrich (St.-Louis, MO, USA), and microwave extraction (Milestone S.r.L., Sorisole, Italy). The last preparation step includes filtration and dilution in 0.5 M HNO_3.

The dissolved sediment sample and water sample were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-OES) using a Spectroblue SOP (Spectro

Analytical Instruments GmbH, Kleve, Germany). The operating parameters of the ICP-OES can be seen in Table 1.

Table 1. ICP-OES operating parameters.

Parameter	Value
Plasma	Argon
Power	1400 W
Coolant flow	13 dm/min
Auxiliary flow	1 dm/min
Nebulizer flow	0.75 dm/min

The samples were diluted with 1% nitric acid and the quantification was achieved using element standards (Sigma-Aldrich, St.-Louis, MO, USA).

2.4. GROWTH MEDIUM AND MICROBIAL CULTURE PREPARATION

The enrichment of the mixed bacterial culture was performed in anaerobic conditions by inoculation of 0.5 dm^3 sediment in 20dm^3 LB nutrient (10 g/dm^3 tryptone, 5 g/dm^3 yeast extract and 5 g/dm^3 NaCl, pH 7) containing 15 g/dm^3 glucose. After 96 hours of cell growth, the enriched culture was suspended in fresh nutrient medium (5 g/dm^3 LB + 1 g/dm^3 acetate instead of glucose to avoid fermentative metabolism) to a microbial concentration of 107 CFU/dm^3 and loaded in the anode chamber of the MFC. The process was conducted at 18 ˚C. The initial organic load of the medium is equivalent to COD 464 mgO_2/dm^3.

2.5 MFC CONFIGURATION AND OPERATION

The MFC used in this study was assembled as a cylindrical plastic reactor consists of two chambers separated by Nafion® 424 perfluorinted proton exchange membrane. The cell segments were equipped with the respective sampling and gas/liquid transport ports. The electrodes were 30mm in diameter made of carbon cloth with stainless steel current collectors. They were connected with external electric circuit loaded with 1000 Ohms resistor. The volumes of cathode and anode chambers were 40 dm3. 2 % solution potassium ferricyanide used as catholyte.

2.6 ELECTROCHEMICAL ANALYSES

Metrohm Autolab PGSTAT101 apparatus and NOVA 2.1 software were used fir cyclic voltammetry in potentiostatic mode with the anode and cathode connected as working and counter electrode respectively. The electrical parameters (open circuit voltage, current and resistance) of the MFC were measured by auto ranging digital multimeter Model MY-66.

All measurements are performed in triple and the results presented here are mean values.

All chemicals used in this study were of the highest purity grade available (Sigma - Aldrich). The results presented in the tables and graphics are mean values of three replicates.

3. RESULTS AND DISCUSSION

The initial hypothesis in this study was that electrochemically active microorganisms capable of anoxic respiration would be present in environments with low oxygen concentration, high organic content and the presence of potential alternative electron acceptors to be reduced.

In order to check this suggestion, we identified a near water body, which eventually meets all three criteria.

3.1. SEDIMENT CHARACTERISATION

During the sample collection, the main physical parameters of the lake water were measured *in situ*. The results obtained showed that the lake is characterized by very low concentration of dissolved oxygen varying from 1.4 to 2.2 mg/dm^3 in the different locations. The conductivity and the pH values were relatively high reaching the mean values of 5230 μS/cm and 8.2 respectively (at 18 °C). The higher values of conductivity are indication for high concentration of dissolved organic and inorganic substances.

The TGA results showed intensive mass change in the interval of 30 to 105 °C (Fig. 2, the green line) which corresponds to the water content of the sediment sample. Another distinctive mass change was observed between 260 and 520 °C, which could be addressed to the combustion of the organic compounds. In correlation to that, calorimetry detected increasing heat released from the sample in the same period (the dark blue line on the Fig. 2). According to the results obtained, the organic matter content of the sediment sample was 22.5% of the dry mass.

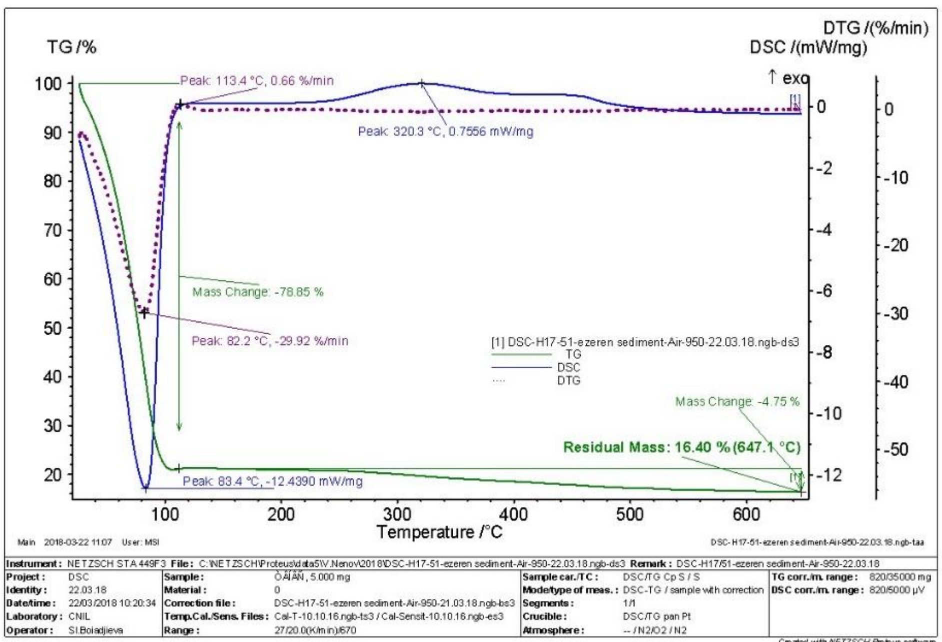

Figure 2. Thermogravimetric analysis data for Lake "Uzungeren" sediments used for estimation of the organic contents

Sediment biodiversity and biochemistry depends on organic carbon content. According to the adopted classification, organic content of about 20 to 25% is a high concentration and it is typical for environments with active sediment metabolism [8]. The biodegradation processes performed by the benthic microbiome are significantly more energy-efficient in the presence of suitable terminal electron acceptors such as dissolved oxygen. However, the lake bottom sediment are usually in anoxic condition and the sediment microorganisms depend on alternative acceptors for their energy and nutritional needs [9].

In order to discover the availability of potential alternative electron acceptors in the studied sediment and water samples, ICP analysis was performed (Table 2).

Table 2. Concentration of main elements present in the sediment and water samples used in the study.

ELEMENT	WATER mg/dm^3	SEDIMENT mg/kg
B	2.151105	112.0807
Mg	775.974	7014.2490
Al	0.018349	8370
Si	2.543293	679.5351
P	0.372954	1537.3662
Cr	Not detected	34.1101
Mn	0.533521	395.0672
Fe	0.763587	30511.8976
Ni	Not detected	271.4268
Co	0.000387	14.4213
Zn	0.009713	124.9506
Cu	0.025476	256.4381
As	0.011003	4.6231
Se	0.023848	2.0683
Cd	Not detected	0.9390

Reducible iron and manganese forms in soil and sediments such as hematite, goethite, ferrihydrite, birnessite and pyrolusite are known to be a subject of microbial reduction and solubilisation [10]. In this sense, the observed high concentrations of Mn and Fe in the tested sediment sample supports the conclusion that anaerobic respiration based on reduction of metal ions is possible in the studied environment.

3.2 BIO-ELECTROCHEMICAL ACTIVITY OF THE SEDEIMENT MICROORGANISMS

Mixed anaerobic bacterial culture was isolated form the collected lake bottom samples. Later, this enriched culture was used as inoculum in the anode chamber of the lab-scale MFC in order to examine the electrochemical activity of the microbial isolates.

The biofilm formation and its electrochemical activity was monitored by the so-called “anode current values” (result of the electron flow from the bacterial cell to the anode surface) which are measured by potentiostatic cyclic voltammetry with specific data processing. The higher the value, better the biofilm performs (Fig. 3.)

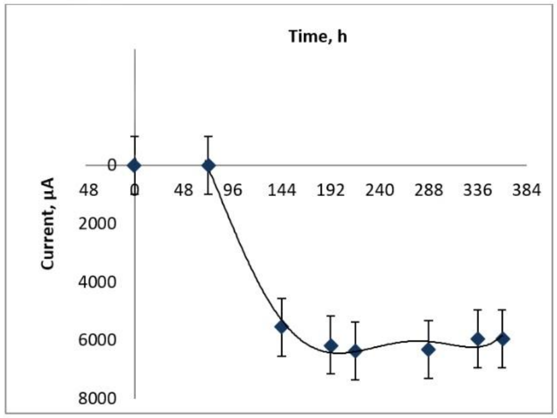

Figure 3. Biofilm development expressed as anodic current measured by the cyclic voltammetry

Starting from zero and after 48 hours of lag phase, the anode current started increasing significantly after the third day of cultivation in the MFC.. This indicates a change in electrochemistry of the anode, probably due to the electrode colonization and biofilm growth. Reaching a plateau after a certain period is associated with the electrochemical stabilization of the system after the biofilm reached a “working” state of development [11, 12 and 13].

The MFC operation was evaluated by the electrical performance and COD removal rate. The open circuit voltage reached its maximal value of 277 mV after one week of cultivation, which correlates with the data for the biofilm formation. During this period, the power density reached 3.5 W/ m^2 (based on the anode surface) and the average COD removal rate observed was 42 mgO_2/dm^3 per day.

4. CONCLUSION

In this study, a lake sediment samples were analyzed in terms of conditions supporting anaerobic respiration. The main goal was to identify the specific physical and chemical parameters of the environments that are associated with this type of metabolism and to define criteria for identification of natural habitats of electrochemically active bacteria (also known as electrogenes). The results demonstrated that this type of organisms are available in the environments with high concentration of organic matter, iron and manganese in combination with low availability of dissolved oxygen. Mixed culture of anaerobic bacteria isolated from the tested sample was successfully implemented to power MFC and demonstrated stable performance in terms of electrochemical behavior and bio-degradation characteristics.

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