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LACTOBACILLUS PLANTARUM AC 11S AS A BIOCATALYST IN MICROBIAL ELECYTOLYSIS CELL

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Abstract. This study demonstrates for the first time the utilization of the bacterial strain *Lactobacillus plantarum* AC 11S as a biocatalyst in microbial electrolysis cell (MEC). The microorganisms were preliminarily immobilized on carbon felt, used as a bioanode in single-chamber membrane-free MEC reactor. The microbial electrolysis was carried out at applied external voltages of 0.8 V and 1.0 V. Experiments with an abiotic control were also performed. At both applied voltages a hydrogen production was registered when a bioanode was used. Cathodic hydrogen recovery of 67 % and 90 % was achieved at 0.8 V and 1.0 V, respectively.

Keywords: microbial electrolysis cell, *Lactobacillus plantarum* AC 11S, biocatalyst, hydrogen production

Introduction

Microbial electrolysis, invented ten years ago (Rozendal & Buisman, 2005), is ecologically clean, renewable and innovative technology for hydrogen production closely related to microbial fuel cell (MFC). Whilst MFCs produce an electric current from the microbial decomposition of organic compounds (Steele & Heinzel, 2001; Liu et al., 2010), MECs partially reverse the process to generate hydrogen (or methane) by applying an electric current (Hu et al., 2008). The anode process of MEC is the same as that of MFC. Although the cathode process in MEC is the same as that of a water electrolyzer (Liu et al., 2010) the operational conditions in both systems are completely different (Liu et al., 2009). MECs typically operate at neutral pHs and at ambient temperatures (20–30^{o}C) suitable for microbial growth.

Microorganisms are one of the most important units in microbiological electrolyser. Both pure and mixed microbial cultures can be used as biocatalysts (Lovely, 2008). Pure

cultures were used mainly in the laboratory studies. Their advantages are: accurately defined microbial composition, known optimal conditions of development, identified specific pathways, etc. However, the use of mixed cultures is much closer to reality conditions (Pham, 2009). The microorganisms may be disposed (immobilized) on the electrode surface, forming bioanode and/or suspended in the electrolyte. These microorganisms have a distinctive feature: they can transfer electrons to a solid electron acceptor, such as an electrode. In MEC, electrochemically active microorganisms oxidize organic substrates on the anode and generate hydrogen on the cathode in the so-called hydrogen evolution reaction (HER). To compensate the negative electrical charge transfer through the external circuit, equivalent quantities of ionic charges are transported between the electrodes inside the cell. The use of microorganisms in electrolysis systems decreases the overpotential, makes easier the electron exchange and decreases the necessary quantity of electricity for the electrolysis. The theoretical cell voltage of MEC (when using acetate as a substrate) is E = −0.11 V, which is much lower than that (-1.23 V) of water electrolysis.

The most investigated microbial cultures in MECs are *Archaea*, *Cyanobacterium*, *Cyanothece* (Bandyopadhyay et al., 2010), dechlorinating bacteria (*Dehalococcoides* spp. and *Desulfitobacterium* spp.) (Lohner & Tiehm, 2009), methanogens and homoacetogens microorganisms (Cheng et al., 2009). *Lactobacillus casei*, *Lactobacillus plantarum* and *Lactobacillus bulgaricus* are among the most widely used strains as lactic acid producers (Reddy et al., 2008). *Lactococcus lactis* has been also used in microbial electrochemical cell experiments (Freguia et al., 2009).

In this study, *Lactobacillus plantarum* AC 11S was explored for the first time as a biocatalyst in single-chamber membrane-free MEC.

Materials and methods

The microbial culture and immobilization

The strain *Lactobacillus plantarum* AC 11S was provided by the Institute of Microbiology at Bulgarian Academy of Sciences. It was isolated from white cheese. The strain was characterized according to the classical phenotypic tests: Gram stain and catalase. Only Gram-positive and catalase-negative cultures with stick shaped cell were selected, which meets the basic criteria for lactobacilli in Kandler and Weis. *Lactobacillus plantarum* AC 11S was isolated as a pure culture on agar medium MRS (DeMan Rogosa Sharp) with pH = 6.5. The optimal temperature for *Lactobacillus plantarum* AC 11S cultivation is 30°C.

The strain was maintained on a semisynthetic medium containing (g/l): yeast extract 10.0; peptone 10.0; sodium acetate 5.0; $MgSO_4.7H_2O$ 0.1; $MnSO_4.4H_2O$ 0.05; agar 20.0; distilled water to 1l.

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The culture was inoculated into semi-synthetic medium containing (g/l): yeast extract 5.5; peptone 12.5; KH_2PO_4 0.25; K_2HPO_4 0.25; sodium acetate 10.0; $MgSO_4.7H_2O$ 0.1; $MnSO_4.4H_2O$ 0.05; $FeSO_4.7H_2O$ 0.05; distilled water to 1l. The pH value was adjusted to 6.8. The culture media were sterilized at 121°C for 20 min. The bacterial cells were transferred from agar slants into the medium and were incubated for 24 h at 30°C in an incubator WiseCube WIS 30, Witeg, Germany. In a conventional fermentation process, 10 ml of the inoculum were added to 100 ml of the culture medium. The fermentation was carried out in flasks without any pH correction for 48 h.

Carbon felt (SPC-7011, 30 g/m^2 Weibgerber GmbH and Co.KG) was used for cells immobilization. The felt was cleaned by washing with acetone:ethanol mixture (1:1) in a ultrasonic bath, was thoroughly rinsed with distilled water and was sterilized at 121°C for 20 min. The sterilized piece of felt was put in 300 ml Erlenmeyer flask (100 ml culture medium, 10 ml inoculum) and the flask was shaken gently at 60 rpm for 24 h to assure cells' adhesion.

MEC construction and operation

The experiments were carried out in a tubular membrane-free MECs. The MECs were constructed from plastic (cylindrical chamber) as a main body with work volume 100 ml and plastic cap, shown in Fig. 1. The cathode and the anode were square-shaped samples with geometric area 9 cm^2 of each electrode. Nonmodified Ni-foam (RCM-Ni-4753.016) was used for cathode. Carbon felt with immobilized cells was used as a bioanode in the single-chamber membrane-free MEC reactor. The anode and the cathode were placed and fixed in special isolated steel holders opposite one another at a distance of 3 mm. MECs were operated at an applied cell voltage of 0.8 V (MEC 1) and 1.0 V (MEC 2), respectively. The electrolysis without microorganisms (abiotic control) was also performed (EC) at $U_{applied}$ = 1.0 V.

The tests were conducted in duplicate at constant temperature 30 ^{o}C. Reactors were operated in a batch mode. The MECs were purged with ultra high purity nitrogen (99.99%) for 30 min and after that they were tightly closed with a plastic cap and sealed with parafilm. Voltage was applied to the MECs by connecting the positive pole of a power supply (Power supply HY 3003) to the anodes and the negative pole to the cathodes. Hydrogen gas was produced in all MEC tests.

Measurement and analysis

A multimeter (MAS TECH MAS-345) was used to monitor the voltage across an external resistor (10 Ω) to calculate current.

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Fig. 1. Photograph of a single-chamber membrane-free MEC reactor

The volume of produced gas by MEC was measured using water replacement method by connecting a gas tight gradual cylinder with the MECs through a plastic tubing.

The cathodic hydrogen recovery $r_{cat} = n_{H_2}/n_{CE}$ of the MECs was calculated. The moles of hydrogen that could be recovered based on the measured current, n_{CE}, is:

$$n_{CE} = \int_0^t Idt/2F$$

where dt (s) is the interval over which data are collected, factor 2 is used to convert moles of electrons to moles of hydrogen and $F = 96485$ C mol^{-1} is the Faraday constant. The moles of hydrogen actually recovered at the cathode n_{H2}, compared to the moles that theoretically could have been produced from the current n_{CE}, is the cathodic hydrogen recovery (r_{cat}) (Cheng & Logan, 2008).

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The amount of lactic acid in the electrolyte was determined at the end of each experiment by neutralization titration. First, a part of electrolyte was centrifuged at 500 rpm for 10 min, after that the supernatant was titrated in the presence of 1-2 drops of phenolphthalein with 0.1 M NaOH. The amount of lactic acid is calculated by using a calibration curve.

Results and discussion

The current densities at different applied potentials of the MECs worked with *Lactobacillus plantarum* AC 11 bioanodes are presented in Fig. 2 together with those for an abiotic control. The current densities obtained with MEC worked in higher applied voltage ($U_{applied}$ = 1.0 V) are bigger than those of MEC 1 ($U_{applied}$ = 0.8 V). The registered values of current density obtained with MECs are several times higher than a control electrolysis cell, worked without microorganisms.

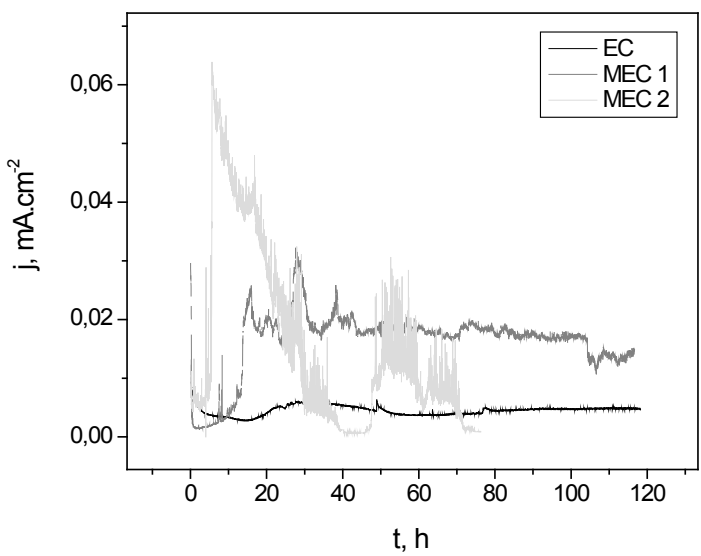

Fig. 2. Development of the current density of both MECs ($U_{applied}$ = 0.8V for MEC 1 and $U_{applied}$ = 1.0 V for MEC 2) and of an abiotic control (EC)

The rates of H_2 production in the begining of the two MECs were very close: for MEC 1: 0.196 ml/h and for MEC 2: 0.212 ml/h (Fig. 3). During the 95-hour yield test, MEC 1 had an average current density of 0.02 mA/cm^2 and produced 8.0 ml of hydrogen, and during the 70-hour yield test, MEC 2 had an average current density of

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0.04 mA/cm^2 and produced 7.8 ml of hydrogen. No hydrogen production was observed in control runs without microorganisms.

The cathodic hydrogen recovery (r_{cat}) of MEC 1 and MEC 2 and the amount of the lactic acid in the end of the experiments are shown in Figs. 4a and 4b, respectively. Although the rates and the yields of H_2 production of the two MECs are very close (Fig. 3), the cathodic hydrogen recovery (r_{cat}) of MEC 2 (90 %) is higher than that of MEC 1 (67 %).

In electrolysis performed at higher potential, $U_{applied}$ = 1.0 V (MEC 2), the amount of lactic acid at the end of the experiment was 3.5 times less than that obtained in the MEC 1 ($U_{applied}$ = 0.8 V). This is probably associated with the more intensive metabolism of the microorganisms under these conditions, as a result of which, after exhaustion of the substrates in the culture medium, they began to consume lactic acid as a carbon source.

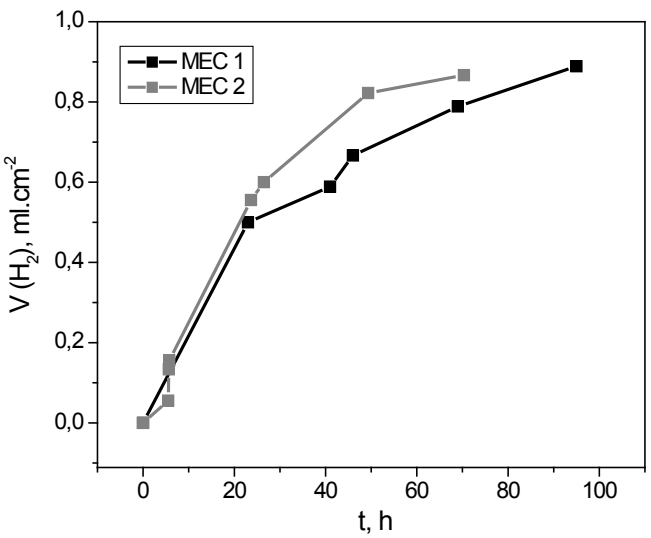

Fig. 3. Time course of H_2 production in batch experiments

Conclusion

In this study we demonstrated the possibility of using *Lactobacillus plantarum* AC 11S immobilized on carbon felt as a bioanode in single-chamber membrane-free MEC. At both applied voltages (0.8 and 1.0 V) hydrogen production was registered. Cathodic hydrogen recovery of 67 % and 90 % and average hydrogen production rates of 0.08 and 0.11 ml/h were achieved, respectively.

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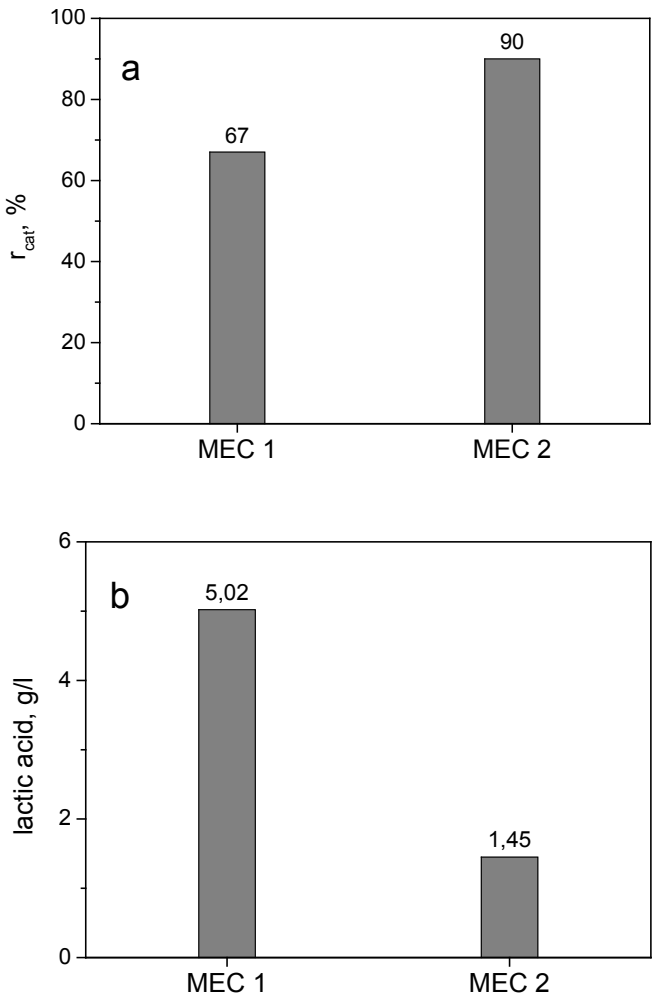

Fig. 4. a) The cathodic hydrogen recovery (r_{cat}) of MEC 1 and MEC 2; b) The amount of the lactic acid in the end of the experiments

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