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Production of low or non-alcoholic beer in microbial fuel cell

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ABSTRACT

Microbial fuel cells (MFC) are bioreactors that are used to generate electricity on the expense of organic substrate oxidation. The MFC's operation-mechanism offers the possibility to use the fuel cell in the beer production technologies to make low or non-alcoholic beers. In this study the effect of MFC anode surface area and riboflavin as an electron shuttle on electric current and on ethanol production in fermentation of wort is demonstrated. The enlargement of anode surface induces an increase of electricity generation (from 4 to 12.5 mA/m^2) while the ethanol production decreases. Adding riboflavin to the wort in the MFC results significant increase in electricity production, furthermore it decreases the ethanol synthesis. Addition of 50 μM riboflavin enhances the current output to 51 mA/m^2 with a 1.5 V/V% decrease of ethanol production, while 100 μM results 86 mA/m^2 and 2 V/V% decrease of ethanol production. These results show the potentiality to brew low or non-alcoholic beer in MFC.
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1. Introduction

Microbial fuel cells (MFC) are applied in the wastewater treatment processes of industrial, agricultural, and municipal wastewaters with the beneficial side effect of generating electric current (Kim et al., 2007). MFC technologies are already successfully used in semi industrial-scales in a brewery (Queensland, Australia) (Zhou et al., 2013) and in a winery (Cusick et al., 2011) as part of a wastewater treatment process (Feng et al., 2008; Wen et al., 2010).

Beer is described as an aromatically sensitive beverage, which means that even minor modifications bring major changes in the aroma profile of beers. The removal of ethanol from the beer heavily affects the sensory properties of the product (Brown and Hammond, 2003). There are two main strategies to produce alcohol free beer one is using physical methods, the other is gaining benefits from the biological processes. The physical methods are based on gentle removal of alcohol from regular beer (vacuum distillation, dialysis, osmotic distillation, etc.) (Liguori et al., 2015). Biological approaches are based on limited ethanol formation during fermentation. The inhibition of ethanol production can be achieved by using special yeast strains (Selecky et al., 2008), and also using continuous fermentation with immobilized (entrapped, enclosed) yeasts (Pilkington et al., 1998), etc. With these biological methods the sensory properties of the low (or non) alcohol beer differ less from the alcoholic homologue than the physically treated beer, such beers are usually off-flavored, because of the aroma-loss (Branyik et al., 2012). Several studies reported the capability of extracellular electron transport of yeast cells in the presence of different mediator molecules like methylene blue, neutral red, riboflavin, etc. in MFCs (Babanova et al., 2011; Rawson et al., 2012). In MFC the reduced NADH molecules are re-oxidized by the reduction of mediators thus the electron transport chain terminates (Prasad et al., 2007). The electrons are transported

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through the microbial cell wall, and the cycle terminates by the oxidation of mediators on the fuel cell anode surface. Therefore the alcoholic fermentation pathway is deactivated in the absence of NADH, and the formation of ethanol is limited (Searle and Kirsop, 1979). In addition many *Saccharomyces cerevisiae* strains can metabolize ethanol as carbon source in sugar and nutrient limited and aerated conditions (Kaliterna et al., 1995). The anode of MFC also serves as electron acceptor this way enhancing the conversion of ethanol to CO_2 through gluconeogenesis. In this study a new fermentation method is demonstrated for low alcoholic beer production. The hopped sweet beer-wort samples were fermented in MFC systems and the effect of MFC redox conditions on the yeast carbohydrate metabolism; ethanol production and electricity production were evaluated. The effect of the size of the MFC anode surface, and the effect of the non-toxic extracellular electron mediator, riboflavin on the electric performance and the ethanol production of yeast were analyzed as well. Using MFC for wort fermentation gives us new possibilities to make low or non-alcoholic beer while generating electricity during the fermentation.

2. Materials and methods

2.1. Inoculum preparation

In the experiment *S. cerevisiae* WS-120 (Hefebank Weichenstephan, München) a bottom-fermenting (lager) yeast was used as fermentative microorganism in the microbial fuel cell. For propagation of the yeast strain YEPD (Yeast extract–Peptone–Dextrose) broth was used at 30 °C.

2.2. Composition of wort

The wort for the experiments was made of pilsner malt without any adjunct. The all-malt wort was brewed with infused mashing that was followed by the mash separation and hop boiling steps (EBC). The original extract content of the beer was 12°B, with bitterness of 25 IBU.

2.3. Construction and operation conditions of MFCs

In the experiment, dual-chamber type of MFCs were applied. In every case approx. 10^7 CFU/mL yeast cell was used to inoculate the anode chamber. Riboflavin was added to the hopped wort, as a non-toxic extracellular mediator in concentrations of 0 μM, 50 μM and 100 μM. Potassium ferrocyianide, 0.1 M, was used as catholyte in the MFC. The working volume of anode and cathode chambers was 12 mL separately, and they were separated with proton-exchange membrane (Nafion 117). Graphite plate, projected surface area of 12 cm^2 (smaller size) and 24 cm^2 (larger size), was used as anode in the MFC settings. Graphite plate was used as cathode in each MFC, having a projected surface of 24 cm^2. The MFCs were operated separately in batch mode, no pre-inoculation was used. After each experiment the MFCs were emptied, cleaned and sterilized. Electrodes were connected to an external resistance (500 Ω) and parallel connected to a digital multimeter. The generated voltage on the external resistance was measured. Using Ohm's law ($I = V/R$) the electric current was calculated from the registered voltage values. The running temperature was 15 °C in the metabolic analysis, in other cases 30 °C. The pH of the anode and cathode chamber was neutral (pH 7.0 ± 0.5). Each experimental run was carried out simultaneously, in triplicates in three independent MFC units to confirm the reproducibility and reliability of the results.

2.4. Control experiment

As a control sample, the same type of wort was fermented with the same yeast strain on 30 °C for 3 days in the MFC device, however the two electrodes (anode and cathode) were not connected to each other. All other conditions were the same as other MFC settings.

2.5. Fermentable sugar concentration measurement

Fermentable sugars of the wort were detected by a HPLC system (Surveyor, Thermo Scientific, San Jose, USA) with an Hi-Plex H column (Agilent, Santa Clara, USA). The mobile phase was 5 mM H_2SO_4 solution. The flow rate for elution was 0.6 mL/min at 45 °C. The measurement time was 25 min at constant flow rate. The carbohydrates were detected by refractometric index (Surveyor RI Plus Detector, Thermo Scientific, San Jose, USA). All chemicals for the standards were HPLC grade of purity and used without further purification.

2.6. Alcohol content measurement

Two different methods were used to determine ethanol content of the samples. In case of metabolism analysis the ethanol concentration was measured by HPLC (same set up as at carbohydrate measurement) with RI detector. In other cases the alcohol content of the beer was measured with Anton Paar 4500 M Alcolyzer system. After 3 days of operation ethanol concentration of the anolyte of MFC was measured. In case of the control samples the fermented wort samples were centrifuged to remove separate sediments from the fluids and cold bath-ultrasound treated to remove the produced CO_2.

2.7. Statistical analysis

Statistical analysis was carried out using the Statistica 8 (Statsoft, USA) software. One-way analysis of variance (ANOVA) for repeated measures was applied to the data obtained from the result of ethanol measurements. Results from treatments showing significant overall changes were subjected to post-hoc Tukey's test with significance for $\alpha = 0.05$ (p-value shows the possibility of making statistical first type error, n is the size of the sample, α is the level of significance).

3. Results and discussion

3.1. Yeast metabolism during the MFC treatment

Wort in the anode chamber of three MFC unit was inoculated with *S. cerevisiae* yeast. The three independent MFC units were run on 15 °C for 2 weeks which is a typical fermentation time in beer fermentation. The sugar consumption throughout the metabolism of the yeast was evaluated by simultaneous monitoring of fermentable carbohydrate concentration (taking samples in every 8 h), ethanol concentration and electricity generation. The measured parameters are shown on Fig. 1.

This kinetic's of current generation is in accordance with the main metabolic pathway regulations of fermentative sugars in yeasts (Pasteur-effect, Crabtree-effect). The performance of electricity production in yeast-based MFC can be significantly influenced by the substrate concentration. At the

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