Elimination of nitrate ions in bio-fuel cells

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Abstract. The article deals with methods for elimination of nitrate ions in the cathode processes of biofuel cells. BFCs based on *Micrococcus luteus* (1-i) isolated from activated sludge of wastewater treatment facilities of Angarsk Petrochemical Plant (Angarsk, Russia) were more efficient under substitution of oxygen (the most typical acceptor of electrons in the BFC technology) with nitrate. During the 97-hour experiment, power values of the oxygen-based BFC reached 113 ± 49 uW, while for the nitrate-based BFC, the value was 450 ± 19 uW. Energy production was accompanied by nitrate reduction in the BFC catholyte.

1. Introduction

Ammonium nitrate and nitric acid widely used in the mining industry pollute nearby water objects [1]. Removal of these ions from wastewater is a serious problem for biological treatment of industrial, domestic and agricultural wastewater. When using traditional biological treatment methods, high concentration of nitrogen is recorded [2]. In this regard, development of methods for elimination of nitrates from wastewater is a crucial environmental task. Over the last years, in the context of depleted energy sources, alternative energy sources have been actively developed [3, 4]. A BFC-based technology is one of the promising development directions [5–9].

The technology solves two major problems - environmental (the need for waste treatment, wastewater treatment, removal of pollutants from various environments) and energy (generation of electrical energy during the bio-oxidation of various compounds). Electrons released from microorganism oxidation of various components of wastewater are transferred to an anode electrode. When an anode electrode closes with a cathode, electrons move from the anode to the cathode [10].

For successful operation of BFCs, electrons have to be transferred from the electrode to the final acceptor. Due to high redox potential and easy production, oxygen is a widely used electronic acceptor in the cathodic process. The studies on oxygen-based cathodes are of great interest due to their high efficiency compared to chemical cathodes [11].

However, most studies show that it is required a large amount of energy to supply oxygen to the cathode chamber which is not economically feasible [11]. Oxygen-based BFCs consume large amounts of energy. Therefore, alternative electron acceptors have to be used [12] (metal ions of variable valence, perchlorate, persulfate, azo dyes, chlorophenols, nitrobenzene, etc.) [11, 13].
The use of alternative electron acceptors can increase energy production volumes, reduce operating costs and expand the scope of BFC application. Since redox properties of nitrate and oxygen are similar, nitrate can be used as an electron acceptor in the cathode chamber [11]. The research purpose was to analyze efficiency of the BFC based on *Micrococcus luteus* (1-i) isolated from active sludge of wastewater treatment facilities of Angarsk Petrochemical Plant (Angarsk, Russia). Nitrate was used as a cathode acceptor of electrons.

2. Material and methods
The design of the BFCs was described in [14]. Ural T-22R A carbon fabric (SvetlogorskKhimvolokno, the Republic of Belarus) was used as an electrode. The size of the electrode was 16 × 4 cm. *Micrococcus luteus* (1-i) was used as an anode bioagent. It was isolated from active sludge of wastewater treatment facilities of Angarsk Petrochemical Plant (Angarsk, Russia). Model wastewater was a main working medium [14]. Sterilization was performed at 1 atm during 45 minutes. Both BFC chambers were sterilized with a 3 % hydrogen peroxide solution during 20 minutes. Then, each BFC chamber was washed with sterile water to remove residual H₂O₂ and placed under UV light for 15 minutes. In compliance with aseptic regulations, in order to remove the air from the chamber, the BFC chambers were filled with model wastewater. In the experiments with oxygen, the cathode chamber was filled so that the water level was 2-3 cm below the upper level of the chamber to allow aeration of the catholyte. Through special holes in the upper part of the BFC, carbon fabric electrodes were placed and fixed with rubber stoppers tightly fitting to Plexiglas. Through a special rubber plug in the side of the chamber, *M. luteus* (1-i) and peptone (0.5 g/l) were introduced into the anode chamber using a sterile syringe. The BFC cathode chamber was aired with argon for 10 minutes to remove oxygen. Then the syringe introduced KNO₃ (0.1 g/l). In the oxygen-based cathodes, artificial blowing was carried out with a Dezzie D-044 compressor (China). The concentration of nitrate ions in the BFC catholyte was measured by the photometric method with salicylic acid according to FERA 16.1: 2: 2.2: 3.67-10. The catholyte was sampled for analysis using a sterile syringe without disturbing sterility and anaerobic conditions in the catholyte [15, 16]. Generated voltage was measured continuously on an automatic basis. Results were recorded using an Arduino Mega 2560 microprocessor board-based system (ATmega 2560).

It allowed simultaneous reading of data obtained from 16 BFCs every 5 minutes of the experiment. The current values were recorded using a DT-266 multimeter (China). Using voltage and current values, BFC power (μW) was calculated: \( P = U \times I \). Statistical processing of experimental data was carried out using the Microsoft Office software package. Conclusions were made with probability \( P > 0.95 \).

3. Results and discussion
Experiments identified that NO₃⁻-based BFCs are more efficient than oxygen cathode-based BFCs. During the 97-hour experiment, oxygen cathode-based BFCs generated voltage up to 193 ± 12 mV for 97 hours. The current value increased to 782 ± 17 μA. Nitrate-based BFCs generated voltage up to 402 ± 30 mV, and the current value was 1140 ± 44 μA (Figure 1).
Figure 1. Dynamics of voltage (a) current (b) generated in oxygen and nitrate-based BFCs (an anolyte and catholyte medium is model waste water; a bioagent in the anode chamber is *M. luteus*, a medium for microorganisms is peptone (0.5 g/l); a source of nitrate ions in the catholyte is potassium nitrate (0.1 g/l), oxygen is supplied by a compressor). ■ – an electron acceptor in the cathode is nitrate; × – an electron acceptor in the cathode is oxygen.

The maximum value of power generated in the BFC was calculated. The data identified an increase in power characteristics of the nitrate-based BFCs in comparison with the oxygen-based BFCs. Thus, oxygen as an electron acceptor helped increase generated power up to 113 ± 49 μW, while substitution of oxygen with nitrate increased power four times (450 ± 19 μW) (Figure 2).

Figure 2. Comparison of the highest values of power generated in the oxygen and nitrate-based BFCs (an anolyte and catholyte medium is model waste water; a bioagent in the anode chamber is *M. luteus*, a medium for microorganisms is peptone (0.5 g/l); a source of nitrate ions in the catholyte is potassium nitrate (0.1 g/l), oxygen is supplied by a compressor).
Thus, substitution of oxygen with nitrate in the BFC cathode chamber increased electrical properties of the BFCs.

The dynamics of concentration of nitrate ions in the BFC cathode chamber was studied. During the 97-hour experiment, the nitrate content in the BFC cathode decreased by 55 ± 0.36%. That speaks for the use of nitrate as an electron acceptor in the BFC cathode chamber (Table 1).

Table 1. Dynamics of the nitrate ion concentration in the BFC cathode (an anolyte and catholyte medium is model waste water; a bioagent in the anode chamber is *M. luteus*, a medium for microorganisms is peptone (0.5 g/l); a source of nitrate ions in the catholyte is potassium nitrate (0.1 g/l).

| Duration, h | Concentration of nitrate ions in the BFC cathode chamber, mg/dm³ |
|-------------|---------------------------------------------------------------|
| 0           | 2.89 ± 0.07                                                   |
| 24          | 1.62 ± 0.04                                                   |
| 97          | 1.31 ± 0.02                                                   |

In order to prevent the results of nitrate content dynamics analysis from being distorted, permeability of the MF-4SK ion-exchange membrane for NO₃⁻ ions was studied. To this end, KNO₃ (0.1 g/l) was added to one of the BFC chambers containing distilled water. Within 72 hours, the concentration of nitrate ions was determined in both chambers. Based on the experiment, it was identified that the MF-4SK ion-exchange membrane is nitrate-ion impermeable. Thus, during the 72-hour experiment, the amount of nitrate ions diffusing through the BFC membrane was only 1.04 ± 0.05%.

4. Conclusion
Thus, along with microorganism oxidation of a wide range of wastewater components in the BFC anolyte and current generation, NO₃⁻ ions can be eliminated in the BFC catholyte. This process consumes electrons entering the BFC cathode from the anode chamber when the circuit is closed. The use of nitrates as electron acceptors can reduce operating cost, since oxygen supply to the cathode chamber is an energy-intensive process. Therefore, nitrate introduced into the catholyte is eliminated which increases energy generation. The use of nitrate-based BFCs is a promising technology which can be successfully applied for biological wastewater treatment.

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