Redox-active molecules in bacterial cultivation media produce photocurrent

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Abstract

In recent years, global climate change has urged the importance of developing new clean energy technologies that may replace the combustion of fossil fuels and help to reduce carbon emissions. A promising method is the utilization of bacteria as electron donors in microbial fuel cells. In this work, we show that the cultivation media of bacteria that is based on yeast extract consists of redox-active molecules that can be used as electron donors reducing the electron acceptor Cytochrome C and producing an electrical current in a bio-electrochemical cell. We apply cyclic voltammetry and 2-dimensional fluorescence spectra to identify the redox active molecules NADH and Flavines that may play a key role in the electron donation mechanism. Finally, we show that upon illumination, the current production is enhanced.

Keywords: Bacteria, Yeast extract, Cultivation media, Fluorescence, Electrical current, Photocurrent.

Introduction

For many years, the combustion of fossil fuels has been a major source of energy production around the world. The realization that massive carbon emission triggers climate changes that may lead to a global disaster in the future, has urged the development of novel clean energy technologies. Among the leading technologies that dominate the energy market are solar cells[1], turbines[2], and hydro plants[3]. The promising concept of microbial fuel cells (MFCs) was invented more than a hundred years ago by Potter et al[4]. In this method, bacterial cells are integrated with an electrochemical cell and applied as electron donors at the anode. Bacteria can perform external electron transport (EET) via 2 kinds of mechanisms: A direct electron transfer (DET) [5–7] and a mediated electron transfer (MET) [8–13]. DET is conducted by trans membranal conductive protein complexes such as metal respiratory complexes [14,15] or conductive *pili* [16–19]. The bacterial species *Shewanella oneidensis* [17] and *Geobacter. Sulfurreducens* [20] that are rich in these complexes perform a high DET-based exo-electrogenic activity. Natural MET is done by the secretion of electron donors from the cells such as NADH[21] Phenazines and Quinone derivatives [8–13]. The electrical current production may be enhanced upon the addition of exogenous electron mediators [22–27]. One of the expenses that is required for the bacterial viability in MFCs is a continuous supplement of a cultivation media. This can be bypassed by using photosynthetic microorganisms that can synthesize their own carbon source by conducting photosynthesis. [28,29]. Another applied method is plant-based MFCs. In this approach, organic material is released from degraded plant roots and can be used for bacterial cultivation in the soil. Regardless of the soil bacteria, roots can also perform EET[30] and apply as electron donors in bio-electrochemical cells[31]. The current production from bacteria can be enhanced using a biofilm architecture that is in close association with the anode[32]. MFCs are not limited to bacteria and can use additional species such as yeast[33]. Furthermore, the variety of live organisms as electron donors in BECs has been recently broadened to macro-organisms such as seaweeds and plant leaves[34–37].

In addition to using whole organisms, isolated components may also apply for a bio-electricity generation. Among these components are enzymes that can apply as catalysts in BECs[38–42]. Another approach is to use isolated photosynthetic components such as Photosystems[43], Chloroplasts[44], and Thylakoid membranes[45] that upon illumination can perform oxygenic photosynthesis to extract electrons from the water and reduce an exogenous electron mediator or directly reduce the anode of the BEC.

In this work, we show that the bacterial cultivation media Luria-Bertani (LB) which is based on yeast extract consists of reducing molecules that can be used for the electrical and photoelectrical current generation. We identify some of these molecules as NADH and Flavins.

Results and Discussion

Luria-Bertani media consists of reducing components

Previous studies have reported the utilization of the protein Cytochrome C (Cyt) as an indicator for the presence of reducing molecules in a solution [31]. The absorption spectrum of fully oxidized Cyt shows a single peak at λ=475 – 600 nm with a maximum at 530 nm. Upon reduction of Cyt, a new peak appear with a maximum at 550 nm. The intensity of this peak correlates with the number of reduced Cyt molecules in the solution. To explore whether LB media consist of reducing molecules, A solution of 30 µM Cyt was incubated with LB. The absorption spectra of Cyt were measured right before the incubation and after 30 min. The obtained spectra showed a peak with a maximum at 550 nm that whose intensity was increased in correlation with the incubation time (Fig. 1).

Fig.1 - Luria-Bertani media consists of reducing components. Absorption of LB fully oxidized Cyt and a mixture of Cyt +LB after 30 incubation. The intensity increase at 550 nm indicates Cyt reduction [31] Absorption spectra of LB (black), Cyt (red), and Cyt + LB (blue). **b** Average absorption at 550 nm of LB (black), Cyt (red), and Cyt + LB (blue). The error bars represent the standard deviation over 3 independent repetitions.

Studying the electric potentials of redox-active components in Luria-Bertani media.

Following the observation that LB consists of molecules that are capable of charge transfer, we wished to characterize the potential in which these components can accept or donate electrons when integrated with an electrochemical cell. To do this, we used screen printed electrodes (SPE) (Basi, SP- 1401) with a Graphite working electrode (1mm in diameter) and Counter electrode, and silver coated with silver chloride reference electrode). A drop of 50 µL of LB was gently placed on the SPE. Cyclic voltammetry (CV) was measured (0 – 1.2 V Scan rate = 0.1 V/s, 5 cycles). The obtained results showed positive peaks with maxima around 0.9 V whose intensity was decreased and correlated with the cycle number (Fig. 2a). This voltammogram fingerprint correlates with the fingerprint of NADH[46].

Fig.2 - Studying the electric potentials of redox-active components in Luria-Bertani media. CV of LB was measured at potentials 0 – 1.2 V, scan rate 0.1V/s. Cycle 1-5 (black, red, blue, green, and purple respectively).

Identification of redox-active molecules in Luria-Bertani media.

Previous studies have shown that redox-active molecules such as NADH[21] and Flavines[17] may play a major role in mediating electrons between live organisms and the anode of BECs to produce current. These molecules can be selectively identified in complex matrixes based on their unique 2D-fluorescence spectral fingerprints [47]. To study whether such molecules exist in LB, we applied 2D-fluorescence maps (λ_{Ex} = 230 – 600 nm, λ_{Em} = 250 – 800 nm). The obtained spectra showed a main peak with maxima around (λ_{Ex} = 300 nm, λ_{Em} = 450 nm), (λ_{Ex} = 360 nm, λ_{Em} = 450 nm) and a shoulder with maxima around (λ_{Ex} = 450 nm, λ_{Em} = 510 nm) that correspond to the fluorescent fingerprint of NAD$^+$, NADH and

Flavines[21,47]. Based on the CV measurements (Fig.2) and the 2D-fluorescence maps. We postulated that NADH in LB may be a major source for current production in a BEC.

Fig.3 - Identification of redox-active molecules in Luria-Bertani media. 2D - fluorescence spectra of LB (λ_{Ex} = 230 – 600 nm, λ_{Em} = 250 – 800 nm) were measured to identify the presence of NAD^+, NADH, and Flavines.

Luria-Bertani media produces current and photocurrent in a bio-electrochemical cell.

Based on the observation that LB consists of redox-active molecules, we wished to estimate its ability to apply as an electron donor in a BEC. Furthermore, we wish to explore if some of the molecules that exist in LB can produce photocurrent under illumination. To address these questions, Chronoamperometry measurements of LB were measured in dark/light intervals of 100 s. A potential of 0.9 V was applied to the anode. This potential was chosen based on the peak that was obtained in the CV measurements (Fig. 2). The illumination was conducted using a white LED light (100 W / m^2). 0.1 M NaCl solution was measured as a control. The LB solution produced a current density of ~1.2 µA/cm^2 that was enhanced to 2.4 µA/cm^2

under illumination. We postulated that the photocurrent may originate from molecules in the LB that can perform Förster energy transfer to the anode.

Fig.4 - Luria-Bertani media produces current and photocurrent in a bio-electrochemical cell.
Chronoamperometry of LB was measured in dark and under white light illumination applying dark/light intervals of 100 s.

Suggesting a model for the current and photocurrent production

While LB is based on yeast extract, it consists of numerous molecules classes of molecules that originate from the internal content of yeast such as various proteins, lipids, metabolites, and more. We suggest that rather than NADH and Flavins that were detected in the LB solution, it is possible that many unknown components such as redox-active proteins, vitamins, or quinones may contribute to the current or photocurrent production (Fig. 5).

Fig.5 - Suggesting a model for the current and photocurrent production. NADH, Flavins, and other possible electron donors that originate from yeast extract donate electrons at the anode of an electrochemical cell. These electrons flow further to the cathode and finally to an electron acceptor that may also originate from the yeast extract component in the LB. Upon illumination, Flavin, NAD$^+$, and NADH may absorb the light and conduct Förster energy transfer to the anode.

Conclusions

In this work, we show that the bacterial cultivation media LB consists of reducing molecules that can apply to produce current in a BEC. We further show that upon light illumination, this current is enhanced. We identify some of the reducing molecules to be NADH and Flavines. This discovery may improve the efficiency of bacterial-based MFCs by using both the bacteria and the cultivation media to produce electrical current.

Experimental Section

Materials

All chemicals were purchased from Merck.

LB media preparation

10 g Tryptone, 5 g yeast extract, and 10 g NaCl were dissolved in 1L double distilled water.

Absorption measurements

Absorption measurements of were done at 750 nm (Nanodrop 2000 UV-Vis spectrophotometer, Thermo Fisher Scientific).

2D – FM measurements

The 2D-FM was conducted using a Cary Eclipse fluorimeter (Varian) with excitation and emission slits bands of 5 nm, applying of a voltage of 700 V on the photomultiplier detector.

Chronoamperometry measurements

Chronoamperometry measurements were done using palmsens4 potentiostat with screen printed electrodes (Basi), with a Graphite working electrode (1mm in diameter), Graphite counter electrode, and silver coated with silver chloride reference electrode. Illumination of the LB was conducted from above using a white LED.

Cyclic voltammetry measurements

Cyclic voltammetry was done using palmsens4 potentiostat with screen printed electrodes (Basi), with a Graphite working electrode (1mm in diameter), Graphite counter electrode, and silver coated with silver chloride reference electrode. 5 cycles were measured at a window of 0 – 1.2 V with a scan rate of 0.1 V/s.

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Some of the figures were prepared using Biorender.com

Conflicts of interest

There are no conflicts to declare.

Author contributions

YS conceived the idea. YS and AC designed the experiments. YS performed the main experiments. MS assisted performing the experiments. YS and AC wrote the manuscript. YS and AC supervised the entire research project.

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