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Novel method for screening microbes for application in microbial fuel cell

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HIGHLIGHTS

- The correlation between growth and iron-reducing capacity was observed.
- Regression model (changes of bio-current) was obtained.
- Novel, fast method was developed for screening microbe for application in MFC.

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ABSTRACT

The ability to produce and to transport exo-electrons by microbes either to external acceptors or to electrodes are reported in our study. All investigated microorganisms (exception of *Lactobacillus plantarum*) exhibited strong iron-reducing capabilities in the absence of mediator meaning production and secretion of exo-electrons to the growth medium. *L. plantarum*, *Saccharomyces cerevisiae* and *Escherichia coli* need an electron shuttle molecule to reduce Fe^{3+} ion. Significant correlation was observed between growth and iron-reducing capacity, as well as between initial cell counts and iron-reducing capacity. Changes of bio-current generated in MFC and iron-reduction were experimentally monitored, and a mathematical model was established by regression analysis. Based on these results, a novel and rapid screening method was developed for the selection of microorganisms for potential application in MFC. The method is based on the measurement of absorbance of bacterial and yeast cultures at 460 nm, providing a robust and high sample throughput approach.

1. Introduction

Microbial fuel cell (MFC) is a promising technology for the production of electricity from numerous raw materials such as natural organic matter, complex organic waste or renewable biomass, and can be advantageously combined with applications in wastewater treatment (Oliveira et al., 2013; Wen et al., 2010). Organic compounds commonly used as electron donors are easily biodegradable, including simple carbohydrates (*e.g.*, glucose and saccharose), starch, low-molecular weight organic acids (acetate, oxalate, fumarate), xylose and amino acids (Hassan et al., 2012). The main advantage of this technology is the application of microorganism(s) as a catalyst to convert organic materials directly into electricity, thus it produces bioenergy even from wastes. It is well known that electron transport chains play an important role in adenosine triphosphate (ATP) synthesis where a series of compounds are involved transferring electrons from an electron donor to an electron acceptor *via* redox reactions. Organic matters generally act as electron donors, whereas many electron acceptor compounds (both organic and inorganic) are known. If oxygen is available (*i.e.*, in aerobic respiration), it is used as the terminal electron acceptor, because oxygen provides the greatest Gibbs free energy difference. Moreover, it produces the highest level of energy and microbes usually maximize their energy gain by selecting electron acceptor with the highest potential (Rabaey et al., 2007). In anaerobic environments, different electron acceptors are used, including nitrate, nitrite, ferric iron, sulfate, carbon dioxide, and small organic molecules such as fumarate. Many microbes, for examples iron-reducing bacteria (*Geobacter sulfurreducens*, *Geobacter metallireducens*, *Geobacter toluenoxydans* (Caccavo et al., 1994), *Rhodoferax ferrireducens* (Chaudhuri and Lovley, 2003), *Shewanella putrefaciens* (Kim et al., 2002), *Shewanella japonica*, *Shewanella algae* (Ivanova et al., 2001), are reported to use soluble or insoluble metals or metal-oxides as the electron acceptor, and they are able to transfer electrons out of the cell membrane (Luu

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and Ramsay, 2003). Only those microorganisms can be potentially used in MFC that are able to transfer extracellular electrons to electrodes. Electron transfer can occur either through membrane-associated components (Logan and Regan, 2006; Aelterman et al., 2006), or soluble electron shuttles generated by specific bacteria (Logan and Regan, 2006; Bond and Lovley, 2003) or highly conductive nanowires (Reguera et al., 2005).

The electron transfer mechanism of the iron-reducing bacteria is represented mostly by *Geobacteria* spp. It is usually direct and independent from the type of electron acceptors (Fe(III)-oxide, Fe(III)-citrate, etc.) or different kinds of electrodes (Stams et al., 2006; Feng et al., 2013). For most microbes, the transfer of electrons to electrodes is inefficient because of electrically non-conductive cell walls and impedance by the peptide chain adjoining the active redox center of proteins (Kim et al., 2002). Some special bacterial strains can produce electron mediators and some of them are capable to transfer electrons directly (Chaudhuri and Lovley, 2003; Park and Zeikus, 2000; Pizzariello et al., 2002), therefore such inefficiency can be avoided. Also, another method may be the addition of artificial electron shuttle molecules (mediator). Yeasts generally need a mediator such as methylene blue, methyl orange, resazurin, *etc*., for electron transport (Watanabe et al., 2009; Rahimnejad et al., 2011); accordingly, the application of yeasts in MFC is still very limited.

The operation efficiency of the MFC can be influenced by numerous factors such as microorganisms, design of MFC, type of proton exchange membrane (PEM), type of electrodes, *etc.* (Feng et al., 2013). Among these, the microbial factor is crucial, so the screening of the potential species is essential. Several studies demonstrated different methods to screen the electrochemically active bacteria (*e.g.*, U-tube MFCs (Yu et al., 2012), micro-fabricated MFC arrays (Hou et al., 2009) or Tungsten-oxide nanocluster probe (Yuan et al., 2013). However, these methods take relatively long time (5–6 days) to provide quantitative information about the extracellular-electron production of the microbes. Another limiting factor of these methods is the requirement of expensive equipment and materials; therefore there is an increasing demand for novel and rapid screening methods both in research and in development of MFCs. The main aim of this study was to develop a simple, affordable and high sample throughput method for the screening of microorganism strains for MFC application.

2. Methods

2.1. Microorganisms

G. sulfurreducens DSMZ 12127, *G. toluenoxydans* DSMZ 19350, *Shewanella algae* DSMZ 9167, *Shewanella woodyi* DSMZ 12036 and *Shewanella xiamenensis* DSMZ 22215 were purchased from Deutsche Sammlung von Mikroorganizmen und Zellkulturen (DSMZ), Braunschweig, Germany. *Escherichia coli* ATCC 8739 and *Lactobacillus plantarum* 2142 strains were obtained from University of Perugia, Italy. The yeast strain was *Saccharomyces cerevisiae* WS-120 (Hefebank Weihenstephan, TUM, Freising, Germany).

2.2. Inoculum preparation

Geobacter strains were propagated in *Geobacter* medium DSMZ 579 at 30 °C under strict anaerobic conditions. For inoculation, *Shewanella* strains, *E. coli* and *L. plantarum* bacteria were grown on Marine agar and in Luria–Bertrani (LB) broth at 30 °C and aerated by shaking with 200 rpm. The *S. cerevisiae* strain was propagated in YEPD broth (Yeast extract-Peptone-Dextrose) at 30 °C and aerated by shaking at 200 rpm.

2.3. Iron(III)-reduction assay

To evaluate the microbial reduction of iron(III) ions different broths were applied that were supplemented with the oversaturated solution of Fe^{3+}-citrate as the electron acceptor material (5 g/L). In the case of the yeast strain methylene blue was used as an electron shuttle (mediator). To provide the sole electron-acceptor role of iron(III) ions oxygen was excluded with liquid paraffin on the fluid surface incubated at 30 °C.

Samples were taken each day up seven days. The pH of samples was adjusted to pH 2 with cc. sulfuric acid, and the oversaturated solution of ammonium-thiocyanate (NH_4SCN) was added as a coloring agent (50 g/L). The final dilution factor of the sample was 200 times. After rigorous mixing the absorbance was measured in the range of 300–600 nm by a spectrophotometer to cover the absorbance maximum of the iron(III)–thiocyanate complex.

2.4. Construction and operation conditions of MFCs

For the experiment three identical dual-chambered catalyst-less MFCs were used with methylene blue (300 μM) (Rahimnejad et al., 2011) mediator on the anode side and 0.1 M potassium-ferrocyanide (Wei et al., 2012) on the cathode side. The working volume of anode and cathode chambers was 12 mL, and they were separated with a proton-exchange membrane (Nafion 117, Sigma–Aldrich, USA). Graphite sheets, with a projected surface area of 8 cm^2, were used both as anode and as cathode in all MFC settings. The electrodes were connected to an external resistance (500 Ω) and parallel connected to a digital multimeter (VC-820, Voltcraft, Germany). The generated voltage on the external resistance was measured and the related electric current was calculated. The capacity of the fuel cell was calculated with the $Q = \int I \cdot dt$ equation.

The MFCs were operated in batch mode using the different types of media cultured with 10% inoculum concentration. The composition of the media (LB and YEPD) was the same as above, but the electron acceptor materials (iron(III) ions, oxygen) were omitted.

2.5. Statistical analysis

In order to enhance the data evaluation several statistical methods were performed. The correlation between independent variables was tested by Pearson correlation test. Also two- and three-dimensional linear models were performed to mention the connections of the variables. The models were created by Statistica 8 (StatSoft Inc., USA) software package. The goodness of the fitted model was evaluated by regression analysis, evaluation of root mean squared error (RMSE).

3. Results and discussion

The iron(III)-reduction capability of different microorganisms were tested both in the presence and in the absence of methylene blue as the mediator. The results are shown in Table 1.

With the exception of *L. plantarum* all investigated microorganisms exhibited strong iron(III)-reducing capabilities in the absence of mediator compounds, which indicates the production and secretion of exo-electrons into the growth medium. Generally, *Geobacter* species and *Shewanella* species are iron-reducing bacteria (Weber et al., 2006) thus our results regarding *S. algae*, *S. woodyi* and *S. xiamenensis*, as well as *G. metallireducens*, *G. sulfurreducens* and *G. toluenoxydans* were completely fitted to their capability of the exo-electron production. The *L. plantarum* strain could only reduce the Fe^{3+} ions when methylene blue was present, which means that