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Short communication

Metamorphosis of pathogen to electrigen at the electrode/electrolyte interface: Direct electron transfer of *Staphylococcus aureus* leading to superior electrocatalytic activity

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ABSTRACT

In this paper, we report that *Staphylococcus aureus* isolated from the rumen fluid can display direct electron transfer on carbon felt electrodes and exhibit enhanced microbial electrocatalysis towards the oxidation of complex substrate like cellulose. The phenomena of direct electron transfer and electrocatalysis were investigated in detail by cyclic voltammentry and chronoamperometry. The electron transfer was closer to perfect reversibility with a peak separation value of only 7 mV at a scan rate of 50 mV/s. The enhanced microbial electrocatalysis towards the oxidation of cellulose revealed the potential of the microorganism for application in microbial fuel cells. The pure cultures of *S. aureus* produced an electrocatalytic current density of 1.4 mA/cm^2 as estimated by long-term chronoamperometry for a cellulose concentration of 20 mM. To the best of our knowledge we report for the first time the use of *S. aureus* for bioelectricity generation with cellulose as a sole source of electron donor.

1. Introduction

Microbial electrocatalysis at electrodes is the critical factor that decides the practical applicability of all microbe–electrode interaction-based systems. The electrical communication between microorganisms and electrodes occurs mainly by two mechanisms viz., direct electron transfer via outer membrane cytochromes and conductive pili (widely referred to as nanowires), and by mediated electron transfer via exogenous or endogenous electron shuttles [1–3]. However, incompetent electron transfers from bacteria to electrodes and/or partial removal of microbially produced electrons at electrodes restrict performance of these systems and limit the possibility of scaling up [4]. Therefore researchers are constantly on the lookout for effective microorganisms that show superior electrocatalytic activity. It is known that animals can digest tough cellulose-based substrates effectively with the help of microorganisms present in their rumen fluid. Hence we have made an attempt to isolate the microorganisms present in the rumen fluid to identify microorganisms with superior electrocatalytic properties.

Cellulose is the most abundant biomass found on the earth making it a promising resource as a biofuel [5,6]. Several approaches are being made to dispose the huge volume of cellulosic wastes generated at fast rates [7–10]. Microbial fuel cells seem to be the most suitable option for the simultaneous degradation of cellulose and generation of bioelectricity. Degradation of cellulose and bioelectricity generation in the microbial fuel cell in the presence of *Clostridium cellulolyticum*, *Geobacter sulfurreducans* and *Enterobacter cloacae* has been reported [11,12]. However microbial electron transfer has not been investigated in detail from the perspective of electrocatalysis.

Biofilm formation is a universally observed bacterial trait and it can be formed on all natural and artificial surfaces. Biofilm formation on electrode substrates represents a fascinating example of microbial development and the beneficial aspect of biofilm formation as a potential energy source is reported in this work. Herein we report that the microorganism *Staphylococcus aureus*, considered as an opportunistic pathogen, isolated from the rumen fluid behaves like a typical electrigen at the electrode/electrolyte interface with superior electrocatalytic activity.

2. Experimental

2.1. Collection of rumen fluid

The rumen fluid of goat was collected from the slaughter house in Karaikudi, Tamil Nadu, India. The collected sample was then filtered through whatman 40 filter paper. The filter cake was discarded safely and the filtrate was collected and stored in the refrigerator at 4 °C. The filtrate was then used for the isolation of microorganisms in the rumen. Aseptic conditions were maintained throughout the experiment.

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2.2. Isolation of pure culture

The pure cultures were isolated by spread plating the serially diluted rumen fluid sample. The predominant colonies were isolated and characterized based on the simple biochemical tests as described in Bergey's manual of systematic bacteriology. The characterization of the isolated culture is initially done with gram staining. Several simple biochemical assays were performed including Catalase test, Oxidase test, IMVIC test (Indole, Methyl red, Voges-Proskauer and Citrate tests) and Nitrate reduction tests to identify the microorganisms [13,14]. *Staphylococcus aureus and Staphylococcus warneri* were isolated as pure cultures from nutrient agar.

S. aureus was subcultured in nutrient agar medium and harvested after 48 h of inoculation and subjected to centrifugation at 5000 rpm for 10 min. The supernatant was discarded and the pellet was resuspended in phosphate buffer solution and used for biofilm formation.

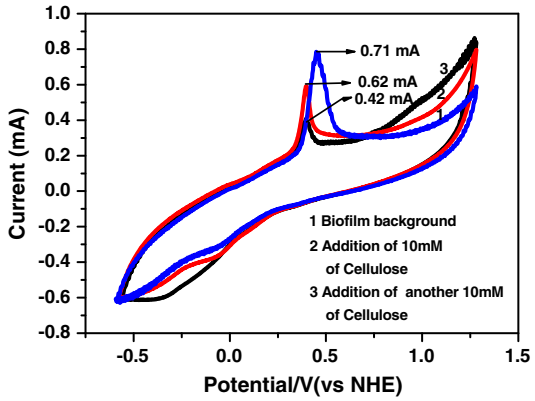

Fig. 1. Cyclic voltammogram of the carbon felt electrode modified with rumen biofilm and the effect of addition of cellulose in phosphate buffer pH = 7.0. Scan rate = 50 mV/s.

2.3. Preparation of biofilm modified electrodes

Carbon felt of dimensions 1 cm × 1 cm (exposed to electrolyte on the two sides and the side walls are insulated, $\phi = 2$ cm^2) was used as the electrode for the electrochemical investigations throughout the studies. The biofilm was allowed to form at open circuit potential conditions on carbon felt electrode in phosphate buffer solution containing cellulose (0.1 g/80 mL of buffer) with 2 mL of the rumen filtrate under anaerobic conditions till a stable potential was reached. The electrode modified with the biofilm was then transferred to an electrochemical cell containing deaerated phosphate buffer solution. Cyclic voltammograms of the electrodes with biofilms were obtained with normal calomel electrode and Pt as reference and counter electrode, respectively. The potential values have been converted to normal hydrogen electrode (NHE) scale in the figures and in the text. Long-term chronoamperometry was performed to evaluate the maximum sustainable current output from the microbial biofilm. Similar method was followed to grow biofilms from the pure cultures of *S. aureus*.

2.4. Morphological characterization of the biofilms

The morphological characterization of the aseptically dried biofilms was carried out with scanning electron microscopy after gold sputtering using the TESCAN equipment.

3. Results and discussion

3.1. Electrochemical investigations with the natural rumen biofilm

The microbial electrocatalysis of the biofilm originating from the rumen biofilm was investigated by cyclic voltammetry. The cyclic voltammogram of the rumen biofilm (Fig. 1) in phosphate buffer recorded under anaerobic conditions exhibited an irreversible redox response with the anodic peak occurring at 0.37 V vs NHE. The electrocatalytic activity of the biofilm was analysed by the addition of 10 mM cellulose. The enhancement in the catalytic current was equivalent to 50 μA/cm^2 for the first addition and 22.5 μA/cm^2 for the second addition of cellulose. The rumen biofilm being enriched by a consortium of microorganisms was able to show enhanced catalytic activity for the oxidation of cellulose. However mixed microbial consortia present in the rumen biofilm was not able to produce a reversible electron transfer at the electrode. The maximum sustainable current obtained from the film was found out to be 0.75 mA/cm^2 by chronoamperometric studies. (Data not shown).

3.2. Electrochemical investigations with the biofilm of Staphylococcus aureus

Cyclic voltammetry was used to study the electrocatalytic behavior of the *S. aureus* biofilm (Fig. 2A). The enlarged version of cyclic voltammetric response of the biofilm on carbon felt electrode is shown in Fig. 2 B. It could be clearly seen that the response was very close to perfect reversibility with a peak separation value of 7 mV at a scan rate of 50 mV/s. The redox response was observed at 0.103 V vs NHE on the anodic side and the reversible cathodic peak occurred at 0.096 V vs NHE. There was another small anodic peak at a potential of 0.206 V vs NHE. The first addition of 10 mM of cellulose led to a huge increase in the current output to 67.33 mA (33.13 mA/cm^2). During the second addition of 10 mM cellulose, the current increased further to a value of 187.60 mA (or 93.80 mA/cm^2). The voltammogram showed the typical hysteresis of a well-behaved bioelectrocatalysis output. It can be seen clearly that in the case of *S. aureus* biofilm, the onset of electrocatalytic activity occurred at a negative potential of −0.22 V vs NHE itself whereas in the case of rumen biofilm the onset of electrocatalysis occurred at 0.34 V vs NHE. The onset of electrocatalysis has been shifted in the negative direction by 0.56 V in the case of *S. aureus* biofilm compared to rumen biofilm. The redox peaks representing the electrical activity of the pure culture (0.103 V) shifted nearly by 0.27 V in the negative direction compared to the rumen biofilm which displayed redox response at + 0.37 V. This observation would have a tremendous impact on the microbial fuel cell applications. A higher negative anode potential of a fuel cell/battery implies greater reducing power of the anode. Also the open circuit potential of the *S. aureus* biofilm modified electrode was always more negative by 100 to 200 mV when compared to rumen biofilm modified electrode indicating higher reducing power of the electrode. Fig. 2C represents the long-term chronoamperogram for the *S. aureus* biofilm. A sustainable current of 1.4 mA/cm^2 was observed in this case.

In bacterial cells, respiratory electron flow occurs at the inner membrane where oxidation– reduction reactions take place in the respiratory chain and the electrons finally exit the outer membrane and reach oxygen which acts as terminal electron acceptor and cellulose acts as electron donor. In the case of microbial electrocatalysis on carbon substrates, cellulose acts as electron donor and carbon substrate acts as terminal electron acceptor and the system is kept under anaerobic conditions. Bacteria must evolve a new mechanism for the transfer of electrons to the solid electrode probably through the cytochrome oxidase enzyme which is the terminal respiratory protein in the case of aerobic prokaryotes. Detailed studies are available on the direct electron transfer of *shewanella oneidensis* and many theories have been proposed to explain the electron transfer from this microorganism [15,16]. Recent analysis of *Shewanella putrefaciens* 200 provided new evidence for an unidentified organic Fe(III) chelator,