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Short Communication

Harvesting energy of interaction between bacteria and bacteriophage in a membrane-less fuel cell

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HIGHLIGHTS

- This study is first to use bacteria–phage interaction for potential generation.
- Potential generation is not due to bacterial lysis by bacteriophage.
- Potential generation does not depend upon bacteria–phage specificity.
- Our microbial fuel cell does not require oxygen.

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ABSTRACT

When a fuel and oxidant flow in laminar contact through a micro-fluidic channel, a sharp interface appears between the two liquids, which eliminate the need of a proton exchange membrane. This principle has been used to generate potential in a membrane-less fuel cell. This study use such a cell to harvest energy of interaction between a bacteria having negative charge on its surface and a bacteriophage with positive and negative charges on its tail and head, respectively. When *Klebsiella pneumoniae* (Kp6) and phage (P-Kp6) are pumped through a fuel cell fitted with two copper electrodes placed at its two sides, interaction between these two charged species at the interface results in a constant open circuit potential which varies with concentration of charged species but gets generated for both specific and non-specific bacteria and phage system. Oxygenation of bacteria or phage however diminishes the potential unlike in conventional microbial fuel cells.

1. Introduction

Microorganisms like bacteria can be used for converting complex organic matter to simple organic and inorganic species such as carbon dioxide, electron and proton. This principle has been used to disinfect organic waste present in water while also generating electrical energy in a fuel cell (Rabaey and Verstraete, 2005; Wang et al., 2013). Fuel cells of this kind, known as microbial fuel cell (MFC), essentially consists of two separate chambers, anode and cathode, for the fuel and the oxidant respectively, separated by a proton exchange membrane (PEM). In a continuo us operation, the microorganisms mixed with the organic material are pumped into the anode chamber where they oxidize the substrate releasing electrons and protons. The electrons get collected at the anode and are transported to the cathode by the external circuit while the protons are transferred through the PEM to the cathode chamber where it accepts the electrons and reacts with the oxygen to produce water (Lovley, 2006). Since their invention, several new designs of MFCs have been developed to increase their efficiencies. The new generation MFCs could be single or double chambered, with or without PEM or a separator (Davis and Higson, 2007; Kim et al., 2008; Lin et al., 2013; Qian et al., 2011). While these MFCs all involve oxidation of an organic nutrient by a microbe, there is no example in which an electrode potential is generated as a result of interaction between two microbial species of different types. This article explores this possibility by bringing in contact a media containing bacteria with one containing the bacteriophage (or phage). Phages attach to the specific receptors on the surface of bacteria, such as lipopolysaccharides, teichoic acids, proteins or flagella, hence infects only certain bacteria bearing the specific receptors, which in turn determines the host range of the phage (Rakhuba et al., 2010). The specific interaction between bacteria and phage has been used in several applications: for eliminating bacterial infection in food items, e.g., that of *Listeria* in poultry foods (Anany et al., 2011), as a therapeutic strategy in which phage is used to kill a targeted bacteria for curing infection (Gupta and

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Prasad, 2011), for protecting live stocks in veterinary, agriculture and aquaculture from bacterial infections (Monk et al., 2010), for enhancing antibacterial effect via conjugation of the capsid protein of phage with strong antibacterial metal particles like silver, for immobilizing phage on biocompatible surfaces like cellulose membrane for variety of purposes (Anany et al., 2011; Cademartiri et al., 2010), for developing biosensors (Singh et al., 2013) and so on.

This study describes the use of facultative anaerobic pathogenic bacteria, the *Klebsiella pneumoniae*, known to cause disease Klebsiella pneumonia leading to lung inflammation and haemorrhage, and the bacteriophage specific to it, to show that it may be possible to harvest energy via their interaction. In essence, media containing bacteria and phage, respectively were pumped through the two inlets of a "Y" shaped microchannel at steady laminar flow. A sharp interface forms between the two liquids at which the two charged species interact which finally result in an open circuit potential. This potential remains a strong function of the concentration of the bacteria and the phage in the respective medium. It is assumed that a charge gradient is formed across each side which finally results in an electrode potential (Arun et al., 2013; Chang et al., 2006).

2. Methods

2.1. Bacterial strain

K. pneumoniae (Kp6) was isolated from human clinical sample (urine) collected from a hospital in Uttar Pradesh, India. Sample was streaked onto the MacConkey agar medium. Lactose fermenting colony was analyzed on *Klebsiella* selective medium. The mucoid magenta colony was subjected to Gram staining, colony morphology and selected biochemical tests (catalase, oxidase, Voges–Proskauer, acid production from glucose, citrate utilization) following the method of Cowan and Steel (Cowan and Steel, 1975). Kp6 is a Gram-negative, rod shaped, lactose fermenting and catalase positive bacteria. Kp6 displayed positive response to Voges–Proskauer, acid production from glucose and citrate utilization tests. The bacterial count was expressed in terms of colony forming units (cfu, number of bacteria per ml of culture). All subsequent bacterial cultures *K. pneumoniae* (Kp6) were done in NZCYM media (Himedia).

2.2. Lytic bacteriophage

To isolate lytic phage, 1 ml log phase *K. pneumoniae* (Kp6) culture in NZCYM broth (pH 7), 10 ml 2X NZCYM broth and 10 ml filter sterilized (0.22 μm, Millipore) sewage water were combined and incubated at 37 °C overnight in a shaking incubator at 100 revolution min^{-1}. Next day, lysate was centrifuged at 5000 revolution min^{-1} (Remi R24) to remove cell debris and clear supernatant was filtered (0.22 μm, Millipore) to obtain bacteria-free filtrate (BFF). BFF was analyzed for the occurrence of lytic phage, if any, by soft-agar overlay method (Adams, 1959). The final titre of phage stock was 10^{15} pfu/ml (plaque forming unit per milliliter of stock).

2.3. Preparation of microchannel

"Y" shaped microchannel was prepared by micro-molding of polydimethylsiloxane (PDMS) (Sylgard 184 elastomer, procured from Dow Corning) mixed with the curing agent (10:1 by weight), on a suitable template (Arun et al., 2013) (dimensions: height, H = 0.3 mm, width, w = 6 mm and length, L = 25 mm) (Fig. 1a). Followed by crosslinking of the polymer, the channel was closed by bonding the solid PDMS block onto a plasma oxidized microscope glass slide. Two thin strips of copper (thickness, 50 μm, size, 0.1 × 3 cm), used as cathode and anode, were connected to a data acquisition card (NI-6009) which was interfaced with a computer to measure the open circuit potential (OCP).

2.4. Experimental procedure

Exponentially grown (~10^9 cfu/ml) bacteria *K. pneumoniae* (Kp6) in NZCYM broth media (pH 7) and phage (P-Kp6, 10^{15} pfu/ml) (pH 7) were injected into two separate inlets of the microchannel using a syringe pump (Harvard Apparatus, USA) at equal flow rates. The Reynolds number $\mathrm{Re} = d_{eq}\nu\rho/\mu$ of flow of both the liquids was maintained low: 0.2–6, so that the flow was essentially laminar. Here, $d_{eq} = 2wH/(w+H)$ represents the hydraulic diameter of the channel, $\nu = Q/(wH/2)$ represents the velocity of each liquid. Q is the flow rate of liquids. ρ and μ denote the density and viscosity of the liquids, respectively.

3. Results and discussion

3.1. Potential generation is a function of flow time but not of the flow rate

In contrast to conventional microbial fuel cells, here two liquids flow in parallel forming a sharp interface at which the bacteria and the phage contact. As a result of the electrostatic interaction between the negative charges on the surface of the bacteria with that of the positive charge on the tail portion of the phage, a potential develops. However, this potential does not develop instantly but over some period of time, during which the open circuit potential of the fuel cell continues to rise, finally reaching a steady state value. The data in Fig. 1b shows that for flow rate varying over an order of magnitude, i.e., Q = 20–600 μl/min, the time required for reaching the final plateau value of potential, E_{max} = 0.29 ± 0.01 volt is found to be τ_E = 90 min. So, neither E_{max} nor τ_E depends on Q suggesting that the observed growth of potential does not result from pressure driven flow of the fuel and oxidant streams.

3.2. Potential generation is not due to bacterial lysis by bacteriophage

The residence time of the liquid (bacteria and phage) inside the channel, $\tau = V/Q$ (V and Q, respectively denotes channel volume and liquid flow rate) was between 2.25 and 0.075 min for the flow rate ranging from 20 to 600 μl. Clearly, such a small residence time was insufficient for the lysis of bacteria to take place, which in a well mixed chamber occurs over 20–60 min (Shao and Wang, 2008).

3.3. Effect of bacteria and bacteriophage concentration on potential generation

In order to find the effect of bacteria and phage concentration on E_{max}, two sets of experiments in triplicates were carried out: in the first set of experiments, NZCYM broth media containing varying concentration of bacteria: 10^3, 10^5, 10^7, 10^9, 10^{11}, 10^{13} and 10^{15} cfu/ml was pumped into the channel, while phage concentration was kept constant at 10^{15} pfu/ml. In the second set of experiments, phage was pumped at different concentrations: 10^3, 10^5, 10^7, 10^9, 10^{11}, 10^{13} and 10^{15} pfu/ml, while the bacteria was maintained constant at 10^9 cfu/ml. These experiments revealed that E_{max} increases with bacterial concentration till an intermediate concentration of 10^9 cfu/ml was reached at which E_{max} was found to be 0.26 ± 0.01 V; for concentration of bacteria exceeding this limit, E_{max} decreases (Fig. 2, line graph with white diamond, ◇). In the other set of experiments, the potential E_{max} was found to increase monotonically with increase in phage concentration, reaching maximum at phage density 10^{15} pfu/ml, at which E_{max} = 0.26 ± 0.04 V was achieved (Fig. 2, line graph with black