



Short Communication

Impact of an external electron acceptor on phosphorus mobility between water and sediments

G. Martins^{a,*}, L. Peixoto^a, S. Teodorescu^{a,b}, P. Parpot^c, R. Nogueira^d, A.G. Brito^a^a Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal^b Faculty of Engineering, Mechatronics and Robotics, University of Valahia from Targoviste, Bd. Carol I, 130024 Targoviste, Romania^c Centre of Chemistry, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal^d Institute of Sanitary Engineering and Waste Management, University of Hannover, Welfengarten 1, 30167 Hannover, Germany

HIGHLIGHTS

- The present study assess the impact of an external electron acceptor on P fluxes.
- Both SMFC tested were able to produce electricity.
- SMFC operation increased metal bound P, Ca-bound P, and refractory P fractions.
- The results indicate an important role of electroactive bacteria in the P cycling.
- This study opens a new perspective for preventing P dissolution from sediments.

ARTICLE INFO

Article history:

Received 29 August 2013

Received in revised form 8 October 2013

Accepted 15 October 2013

Available online 23 October 2013

Keywords:

Lake sediments

Eutrophication

Wastewater

Phosphorus

Sediment microbial fuel cell

ABSTRACT

The present work assessed the impact of an external electron acceptor on phosphorus fluxes between water and sediment interface. Microcosm experiments simulating a sediment microbial fuel cell (SMFC) were carried out and phosphorus was extracted by an optimized combination of three methods. Despite the low voltage recorded, ~96 mV (SMFC with carbon paper anode) and ~146 mV (SMFC with stainless steel scrounger anode), corresponding to a power density of 1.15 and 0.13 mW/m², it was enough to produce an increase in the amounts of metal bound phosphorus (14% vs 11%), Ca-bound phosphorus (26% vs 23%), and refractory phosphorus (33% vs 28%). These results indicate an important role of electroactive bacteria in the phosphorus cycling and open a new perspective for preventing metal bound phosphorus dissolution from sediments.

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1. Introduction

Eutrophication is an environmental dysfunction of surface waters and is the main responsible for the failure in attaining the good water status in 2015 prescribed by the Water Framework Directive (European Commission, 2013). Eutrophication is driven by phosphorus (P) fertilization practices in agricultural areas and by discharges from urban wastewater treatment plants (WWTPs). When ecosystems are eutrophic, all restoration strategies will depend from the success of P removal from the water body, a traditional assumption recently reinforced by Schindler (2012). Therefore, the inputs from the watershed and from the enriched sediments, that were the sink of most P that has been discharged in the water body, should be controlled. Iron (hydr)oxide minerals are potent repositories of nutrients (e.g., phosphate) and the

release of these elements into water column may occur upon dissolution of the iron (hydr)oxides mediated by bacterial processes (Lentini et al., 2012).

Recently, the microbial fuel cell approach has been proposed to upgrade energy inefficient WWTP (Peixoto et al., 2013; Logan and Rabaey, 2012) and sediment microbial fuel cell (SMFC) is composed of a anode electrode embedded in the anaerobic sediment and a cathode electrode suspended in the aerobic water column (Song et al., 2012; Martins et al., 2010). Bacteria in a SMFC mediate the transfer of electrons from carbon sources to the anode thus generating an electric current. In the SMFC anode, the family *Geobacteraceae* is a known anodophile that directly transfers electrons to the anode electrode and other sulfur- or iron-reducing bacteria are classified as electrophiles that indirectly donate electrons to the anode via sulfur and iron redox cycles (An et al., 2013; Sacco et al., 2012; Holmes et al., 2004). Moreover, electroactive microorganisms within *Geobacteraceae* family, that are able to reduce external electron acceptors like iron (hydr)oxides, were already identified in lake sediments (Martins et al., 2011).

* Corresponding author. Tel.: +351 253604412; fax: +351 253604429.

E-mail address: gilberto.martins@deb.uminho.pt (G. Martins).

Therefore, this relation between P fluxes and bioelectricity producing bacteria should be known if a new biotechnological SMFC approach is aimed. This challenge is even more significant since P is a non-renewable resource that should be captured and recycled within a sustainable agro-economy. However, no information is currently available regarding the P speciation in sediments caused by an SMFC operation. Hence, the present work provides the first assessment of the impact of an external electron acceptor and iron (hydr)oxides on phosphorus fluxes. In that regard, a test using a microcosm simulating a SMFC with two types of anodes was carried out and the P concentration was assessed with an adapted P extraction scheme, before and after the system start-up.

2. Methods

2.1. Sampling, analysis, and phosphorus sequential extraction

2.1.1. Sampling and analysis

Sediment samples were collected at the deepest point of Lake Furnas (Azores, Portugal) with a gravitational Uwitec-cover that penetrated about 30 cm in the soft sediments. The sediments were transported to the laboratory (at 4 °C), where the upper 10 cm layer was sliced, mixed, and stored frozen until analysis. The sediments present a close relation with pumice deposits resulted from ancient volcano eruptions, with the granulometry analysis showing a finer fraction composed by silt and clay. Additional characteristics can be found in [Martins et al. \(2012\)](#).

Chemical analysis was performed according to standard methods as described elsewhere ([Martins et al., 2012](#)). Soluble reactive phosphorus (SRP) was analyzed by a spectrophotometric method. Total phosphorus (TP) analysis was achieved by an acid digestion of the samples, with nitric (5 mL) and sulfuric (1 mL) acids for 90 min at 250 °C, and further analyzed as a SRP sample. To determine the organic and water fractions, approximately 0.5 g of sediment was weighed, dried at 105 °C for 24 h and reweighed. Subsequently, the sediment was burned for 2 h at 550 °C and weighed for the quantification of organic components.

2.1.2. Phosphorus sequential extraction

Sequential extraction is much used to evaluate the sediment composition and several processes have been proposed ([Wang et al., 2013](#)). However, the best method depends of the specific goal and three P extraction protocols were tested, namely Psenner ([Psenner and Pucsko, 1988](#)), Golterman ([Golterman, 1996](#)), and Tessier ([Tessier et al., 1979](#)). All extraction methods were tested in triplicate and each extraction step was repeated until the quantity extracted was less than 10% of the previous one with the same reagent. After analyzing all P extraction results, an integration of the three tested methods was done according to [Fig. 1](#). Briefly, distilled water was used to extract labile P, Ca-EDTA, according to [Golterman \(1996\)](#) was used to extract metal bound P, Ca-bound P was extracted using HCl ([Psenner and Pucsko, 1988](#)), organic P was obtained following Tessier protocol ([Tessier et al., 1979](#)), and refractory P was obtained according to Psenner method. This new protocol was applied before and after the SMFC start-up and operation.

2.2. Sediment microbial fuel cell start-up and operation

The bioelectroactivity was assessed in a microcosm experiment simulating a sediment microbial fuel cell (SMFC). Two 1 L open reactors were filled with ~400 g of sediment and 500 mL of tap water. The impact of the anode area was tested using carbon paper (40 cm²) in one reactor and a commercial stainless steel scourer with approximately 812 cm² in the other SMFC. Both SMFC used

carbon paper (Toray) with 40 cm² as a cathode electrode. The anode was buried in the sediment and the cathode was submerged in the water. The electrodes were connected via a 1 kΩ resistor during the first 33 d and via 2 kΩ resistor thereafter. The voltage was recorded by a USB-9215A BNC connector datalogger (National Instruments) and a data acquisition software (Labview 6.0).

Electrochemical analysis was performed according to [Martins et al., 2010](#). The current intensity (*i*) was calculated according to the Ohm's law and the current density (*I*) was calculated as $I = i/A$, where *A* is the projected surface area of the anode electrode. The power density (*P*) was calculated as the product of current intensity and voltage divided by the surface area of the anode ($P = iV/A$). The polarization curve, describing voltage (*V*) as a function of current density (*I*), was recorded using a series of resistances in the range of 41 kΩ–10 Ω. The internal resistance of the SMFC (*R*_{int}) was calculated from the slope of the polarization curve in the region dominated by Ohmic losses ([Martins et al., 2010](#)). The open circuit voltage (OCV) was measured at infinite resistance.

3. Results and discussion

3.1. SMFC start up and operation

The bioelectroactivity of sediment bacteria was determined in SMFC microcosms. Voltage and current density were recorded along operation time and are presented in [Fig. 2](#).

The voltage was very low for the first 18 d, increasing afterwards to a stable value of ~96 mV in the SMFC with carbon paper anode and to ~146 mV in SMFC stainless steel scourer anode, corresponding to power densities of 1.15 and 0.13 mW/m², respectively. This performance might be explained by the gradual formation of an electrochemically active biofilm on the surface of the anode. The open circuit voltage (OCV) was 407 mV in the SMFC with carbon paper anode and ~466 mV in SMFC with stainless steel scourer anode. These values regarding the maximum voltage that it would be possible to obtain in optimal conditions are in line with literature values ([An et al., 2013](#)).

As can be observed in [Fig. 2a](#), the SMFC with stainless steel anode presented a higher voltage. However, the SMFC with carbon anode was more efficient in terms of current density, as shown in [Fig. 2b](#). This was probably due to the higher internal resistance verified in the steel anode, with a lower conductivity and higher inactive area. Another observation is the increment in the voltage after 33 d. This was due to the shift of resistor from 500 to 2000 Ω, based on the resistance where maximum power densities were observed in power curves. In [Fig. 2a](#), it can also be observed a decrease of voltage in the SMFC with stainless steel scourer anode. This was probably due to the increase of internal resistance. The slope of polarization curve showed an increment of 23% from 1150 Ω (33rd d) to 1486 Ω (50th d). The increase of internal resistance might be due to the cathode biofouling which decreased the cathode affinity to oxygen ([Tender et al., 2002](#)). Furthermore, the absence of bulk fluid velocity fluctuations in the current SMFC design increase liquid film resistance and would hamper any increase in the biofilm mass transfer coefficient ([Brito and Melo, 1999](#)). Another possible explanation is mass transfer limitations in the anode. Some authors hypothesized that the deterioration in SMFC performance might be due to organic matter depletion at anode-sediments interface ([Sacco et al., 2012](#)). Indeed, the performance of SMFC is dependent on the microbial metabolism which would be limited by the diffusional transport of soluble organic molecules to the anode ([Holmes et al., 2004](#)).

The polarization curves depicted in [Fig. 3a,b](#) present the voltage as a function of current density obtained during the stable phase of power generation with resistances varying from 41 kΩ to 10 Ω

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