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# ELECTRONIC TRANSFERENCE ASSESSMENT OF THE REDOX PROCESSES AT CARBON ELECTRODES COATED WITH *GEOBACTER SULFURREDUCENS* THAT GROWN AT DIFFERENT TEMPERATURES

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**KEYWORDS**
*Geobacter sulfurreducens*, electrons, cytochromes.

**ABSTRACT**

In the present study, the kinetic parameters of redox reactions and the proteomic profiles of *Geobacter sulfurreducens* grown at different temperatures (25 ºC and 37 ºC) were evaluated. Oxidation peaks with different potentials and current intensities were observed for both cultures. Also, the outer membrane proteins of the bacteria at the two temperatures revealed different profiles that can be responsible for different redox centers. Thus, *G. sulfurreducens* that grows at different temperatures may express different cytochromes in the external membrane.

## INTRODUCTION

*Geobacter sulfurreducens* is a bacteria that can transfer electrons directly to the electrode from different external membrane cytochromes. Each cytochrome is associated with a range of redox potentials, being energetically more favourable than some others (Logan and Regan, 2006). Different bacterial growth conditions,, such as temperature, may influence the prevalence of certain cytochromes in the external membrane (Peixoto et al. 2011). The aim of this work was to evaluate the effect of the growth temperature on the electrochemical behavior of *G. sulfurreducens*.

## MATERIAL AND METHODS

### *Geobacter sulfurreducens* growth

*G. sulfurreducens* (DSM 12127) was obtained from DSMZ (Braunschweig, Germany). The growth of *G. sulfurreducens* was done in 100 ml anaerobic bottles in sterilized microenvironment, at 25 ºC and 37 ºC with sodium acetate as electron donor and sodium fumarate as electron acceptor. Growth medium (826 adapted from DSMZ) was prepared under anaerobic conditions.

### Electrochemical set-up

A thermostated three-electrode glass cell with two compartments separated by an ion exchange membrane (Nafion 117, Dupont de Nemours Co.) was used in the voltammetric study. A saturated calomel electrode was used as reference electrode and a carbon Toray sheet was used as both working and counter electrodes (3x3cm). The effect of the scan rate was studied in order to determined the kinetic parameters of the reactions.

### Proteomic analysis

The *G. Sulfurreducens* outer membrane protein (OMP) extraction was done according to Qian 2009. The first separation of the protein membranes was done by SDS-Page. The complete separation of the membrane proteins was obtained from sucrose gradient centrifugation and 2D electrophoresis. Proteins were visualized by silver staining.

## RESULTS AND DISCUSSION

### Cyclic voltammetry

From cyclic voltammetry studies, it can be concluded that at different temperatures the oxidation peaks potentials and current intensities were different (Figure 1). The current intensity increase when bacteria grow at higher temperature but on the other hand the peak potential becomes more anodic.

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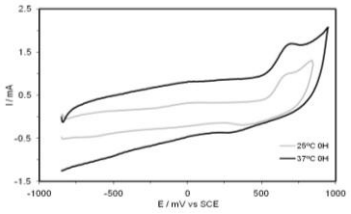


**Fig 1:** Voltammograms of carbon Toray with a suspension of *G. sulfurreducens* that grown at 25 ºC and 37 ºC (50 mVs-1). (room temperature essays)

At lower sweep rates, it was possible to observe two oxidation processes, which are better defined for bacteria grown at 25 ºC.

The oxidation reaction was limited by diffusion. An irreversible electronic transfer is noticed (Figure 2a). At 25 ºC the kinetic of the reaction had a mixed control and the charge transfer was reversible for lower scan rates (Figure 2b).

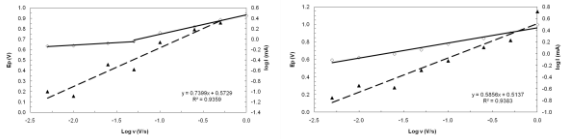

**Fig 2:** log I *vs.* log v (▲) and E versus log v (◊) curves for the oxidation of a pure culture of *G. sulfurreducens* in suspension that grown at 25 ºC (a) and 37 ºC (b).

Comparing the oxidation potentials with literature (Richter et al. 2009), it was possible to conclude that different types of cytochromes can be established as responsible for the heterogeneous electronic transfer.

**Proteomic analysis**

The membrane proteins extracted from bacteria that grown at different temperatures migrated differently in the SDS-PAGE gel, revealing proteins of different molecular weights.

*G. sulfurreducens* may provide an interesting model for structural comparison of proteins since the two samples revealed different profiles, when 100 spots were discriminated (Figure 3). The 2D gel analysis of the outer membrane proteomes at different growth conditions revealed 9 spots in up-regulation and just one spot in down-regulation when bacteria grown at 37 ºC. Future work will address the complete proteins characterization.

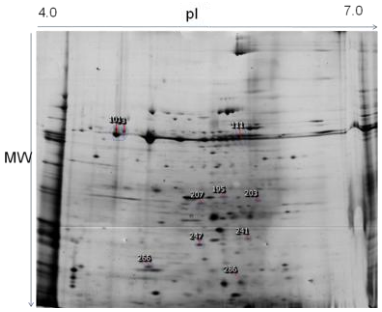


**Fig 3:** 2-DE of OMP of G. *sulfurreducens*

## CONCLUSIONS

The differences observed in voltammetric study can be related to the structural differences in bacteria grown at different temperatures. Changes in the *G. sulfurreducens* growth temperature promotes different protein expression that can be responsible for different redox centers.

## REFERENCES

Logan, B.E. and Regan, J.M. (2006). "Electricity-producing bacterial communities in microbial fuel cells.", *Trends in Microbiology* 14, No.12, 512-518.

Qian, X. (2009). "Investigation of fe(iii) reduction in *Geobacter sulfurreducens* characterization of outer surface associated electron transfer components". PhD Dissertations.

Richter, H.; Nevin, K.P.; Daniel, H.J.; Lowy, A.; Lovley, D.R. and Tender, L.M. 2009. "Cyclic voltammetry of biofilms of wild type and mutant *Geobacter sulfurreducens* on fuel cell anodes indicates possible roles of OmcB, OmcZ, type IV pili, and protons in extracellular electron transfer." *Energy Environ Sci* 2, 506-516.

Peixoto L., Santos A.F.S., Machado I., Sousa A.M., Brito A.G., Parpot P., Pereira M.O., Nogueira R. 2011. "Influence of the Temperature in the electronic transfer mechanism of Geobacter sulfurreducens" In *proceedings of the 3rd Microbial Fuel Cell Conference* (Leeuwarden, Netherland. 6-8 Jun). 145.

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