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Detecting Physiological Status of Microbial Cultures, In-situ

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Abstract

For bioconversion of organic feedstocks to fuels and chemicals to be cost-effective, bioprocesses need to operate at near optimum conditions with sufficient chemical, biochemical and microbial monitoring. To avoid conventional time and labor-intensive monitoring, a new paradigm is required for in-situ, real time analysis. Since bioconversion of organics is accomplished by microorganisms through the oxidation of feedstocks linked to the reduction of electron acceptors, microorganisms can be viewed as electrochemical catalysts. In this regard, following electron flow through well-established electrochemical techniques offers a novel and inexpensive approach to real time monitoring with the advantage of abundant data.

Here we demonstrate the use of electrochemical techniques of cyclic voltammetry (CV) and electrochemical impedance spectrometry (EIS) for monitoring microbial metabolic activity in real time, in-situ. CV provides precise information regarding extracellular electron transfer throughout growth and EIS offers a data rich platform for evaluation of microbial physiological status in real time. In addition, the problem of electrode fouling is managed with voltammetric stripping, an established electrochemical technique used to clean and condition electrodes in-situ.

The effect of organic electron donors as a function of concentration to the physiological status of *Shewanella oneidensis* was determined. In this study, the Gram-negative, pyomelanin overproducer (*S. oneidensis ΔhmgA*) and the pyomelanin deficient mutant (*S. oneidensis ΔmelA*) were chosen due to different surface electrochemical characteristics along with relative degrees of oxygen utilization efficiency. Electrochemical properties changed with growth status and correlated with electron flow from organic carbon sources and terminal electron acceptor availability. These results are compared with those of the Gram-positive *Clostridium phytofermentans* from previous studies.

Introduction

It is important in industrial bioprocessing to control and maintain growth and physiological status of microbial cultures.

Real time in-situ monitoring offers the potential of abundant data, as needed with low costs, potentially leading to automated controls.

Microbes here are regarded not just as biochemical factories but also electrochemical entities.

Microbial cell membranes have an electrical potential difference analogous to a conventional battery that is linked dynamically to cellular activity. For instance, it is the source of free energy enabling such functions as ATP synthesis, pH homeostasis and membrane transport.

Electrochemical cellular processes that change during growth include:

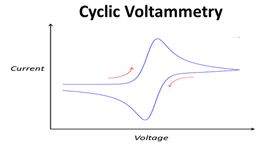
- Membrane potential
- Redox metabolism
- Extracellular electron transfer and/or uptake.

Conventional, well accepted electrochemical techniques provide the opportunity to use in-situ electrodes for real time monitoring.

Biofouling of electrodes can be accomplished through electrochemical stripping techniques.

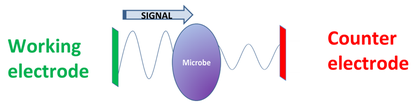
Theory

Cyclic Voltammetry



Cyclic Voltammetry (CV) introduces a cycling potential between two electrodes, then measures the peak current relative to voltage.

Electrochemical Impedance Spectroscopy



Electrochemical Impedance Spectroscopy (EIS) is used here to measure the difference (phase shift and amplitude) of an AC signal sent from the working electrode to the counter electrode . The change in that signal relates to chemical and microbial phenomena during the growth cycle. This approach provides a data rich analyses, especially when measured across a spectrum of frequencies (0.01 – 100,000 Hz). The data were also fit to a circuit model to provide additional information related to microbial growth over time.

Materials and Method

Bacterial strains. *Shewanella oneidensis* exhibits portions of its electron transport chain outside of its outer membrane making it an attractive organism for electrochemical studies. Here deletion mutants were used (Fig. 1). *S. oneidensis ΔhmgA* overproduces the electron shuttle pyomelanin which enhances electron transfer. *S. oneidensis ΔmelA* does not produce electron shuttle pyomelanin and is deficient in extracellular electron transfer.

Growth in Bushnell-Haas broth with 15 g/l glucose was monitored via respirometry to follow glucose utilization and growth coupled to O_2 reduction via CO_2 production and O_2 utilization. Supplemental glucose (15g/l) was added at 48 hours to stimulate additional growth.

Electrochemical studies were carried out with a flat patterned graphite electrode (Fig. 2) inserted into Viton stoppers in the culture vessels. Cyclic voltammetry (CV) scans at 1 V/s from -2 to +2V served for electrode conditioning and cleaning. CV scans a 25 mV/s from -0.8 to + 0.8 V were conducted to determine electrochemical properties of the bacterial suspension, including charge transfer density.

Electrochemical impedance spectrometry (EIS) was conducted from 100K kHz to 10 mHz to monitor signal differences during growth and results in values from the working electrode (real data) and resulting signal passing through the bacterial suspension (imaginary data). Signal frequencies corresponding to membrane potential provide information about cellular physiological status. Imaginary admittance(Y") provides information relative to the cell membrane regarding conductive energy storage/polarization. Cole-Cole plots and Nyquist plots provide information of permittivity (ε) and impedance (Z), respectively.

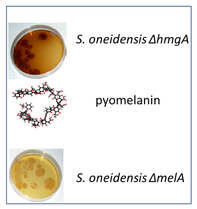


Figure 1. *S. oneidensis ΔhmgA* overproduces the electron shuttle pyomelanin which enhances electron transfer. *S. oneidensis ΔmelA* does not produce electron shuttle pyomelanin and is deficient in extracellular electron transfer.

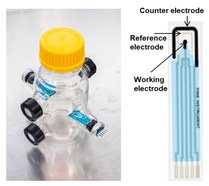
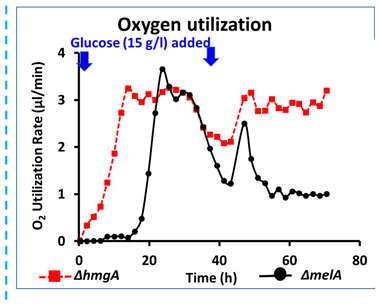
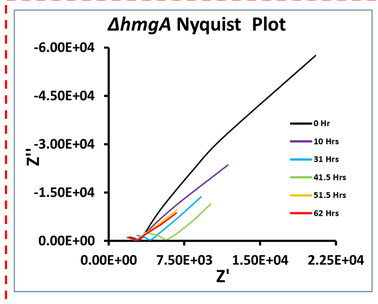
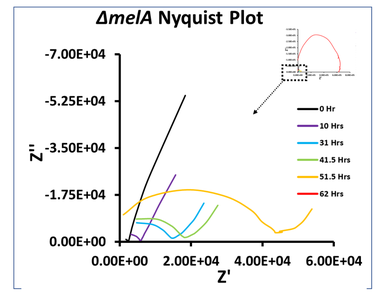


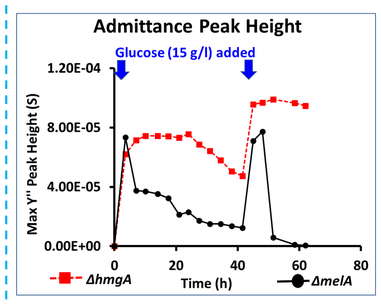
Figure 2. A flat patterned electrode design allowed for analytical continuity and versatility in techniques.

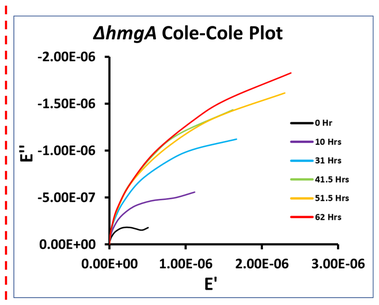
Results

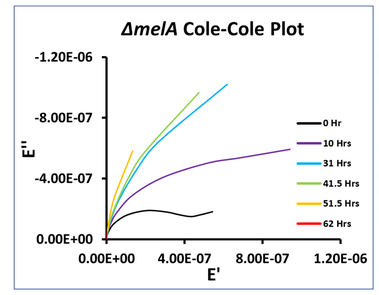


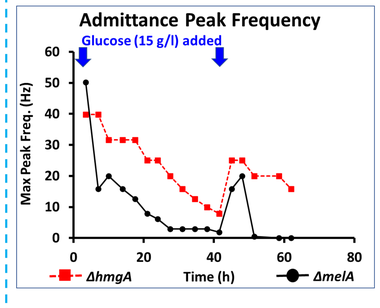


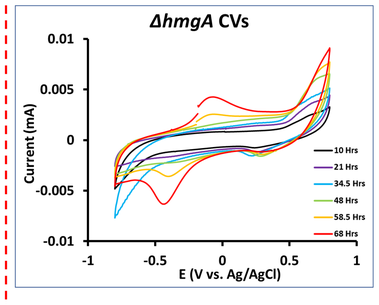


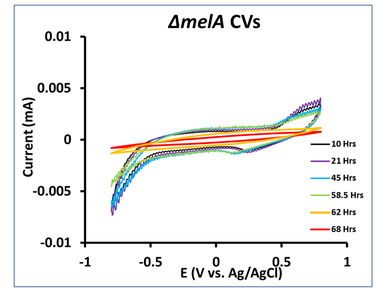


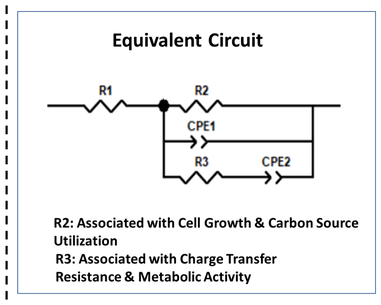


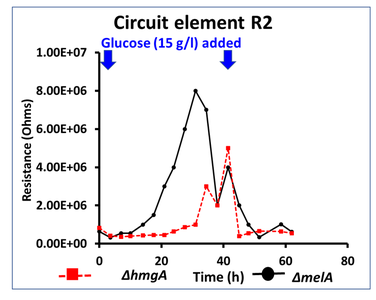


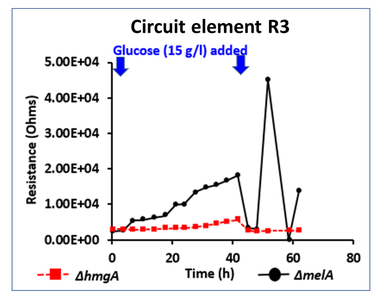












Analysis carried out from frequency range of 63100 – 4.83 Hz

Results/ Conclusions

In-situ electrochemical techniques offer potential to monitor microbial physiological status in real time, as circuit parameters relate to microbial behavior as follows.

· Admittance values (including peak frequency) varied as a function of growth, values of admittance correspond to cell's ability to transfer charge. Admittance decreases as sources of resistance increase.

· CV data provided information about cellular redox status. Loss of defined shape in *S. oneidensis ΔmelA* CVs shows microbes unable to reduce or oxidize the graphite electrode.

· Nyquist and Cole-Cole plots revealed that charge transfer resistance increases as a function of diminished energy source (glucose). Impedance increases drastically as the microbe's ability to grow decreases.

· In contrast to our present work, previously, Gram-positive bacteria demonstrated Cole-Cole plots were indicative of increased charge transfer resistance but not Nyquist plots . This is likely due to cell morphology.

References and Acknowledgements

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