2021

Activity of Saccharomyces cerevisiae by Single Entity Electrochemistry

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Activity of *Saccharomyces cerevisiae* by Single Entity Electrochemistry

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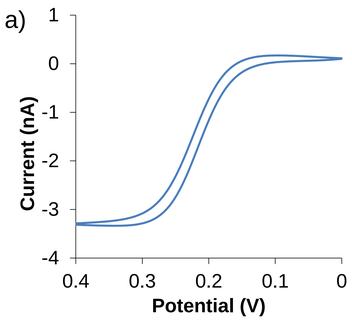
Introduction and Aims

While detection of genes involved in drug resistance has seen major advancements in the past several years, mechanisms of such resistances are difficult to obtain at the moment.[1] Therefore, real-time methods of detection are needed to elucidate said mechanisms and to decelerate microbial resistance to antibiotics. For this research, *Saccharomyces cerevisiae* was selected as a model as they do not travel by chemotaxis like bacteria.[2,3] Detection of individual yeast cells has been achieved through surface area blocking of current generated by the oxidation of ferrocyanide ions at an ultramicroelectrode (UME). COMSOL Multiphysics will be used to elucidate collision dynamics of cells at the UME. In addition, redox cofactors found inside yeast and on their membranes may be detected with mediators[4] and may be distinguishable from the blocking mechanism due to time-scale differences as small molecules have orders of magnitude greater diffusion rates than cells.[5] At a successful completion of these studies, there is potential for the evaluation of antibiotics as well as new information on resistance mechanisms and how they may be circumvented.

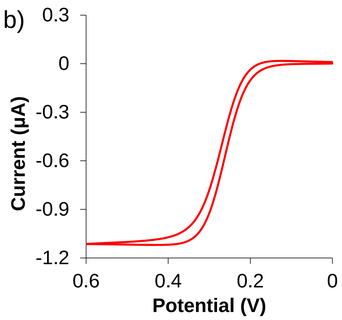
Blocking Experiments

a)



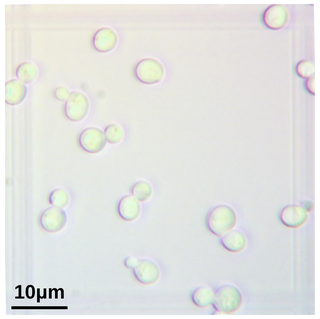
a) Cyclic Voltammogram (CV) of 1mM ferrocene methanol and 100mM KCl to determine diameter of 25µm platinum UME

b)



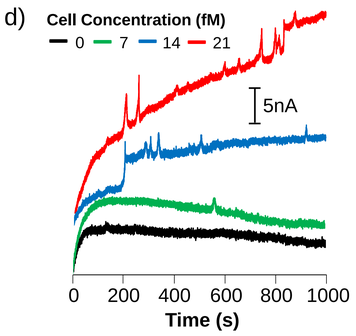
b) CV of 300mM potassium ferrocyanide (KFeCN) and 100mM KCl to determine steady-state potential for blocking experiments

c)



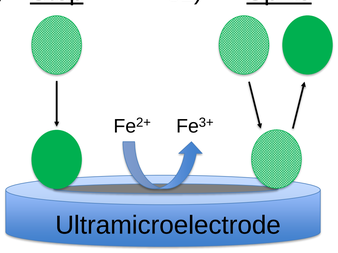
c) Microscopic image of *Swe1Δ Saccharomyces cerevisiae* with 100x magnification. Hemocytometer is used to determine concentration of cells

d)



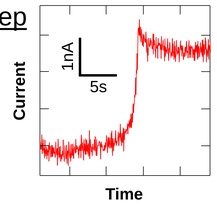
d) Current vs Time plots of 300mM KFeCN and 100mM KCl with increasing concentration of cells

e1) Step e2) Spike

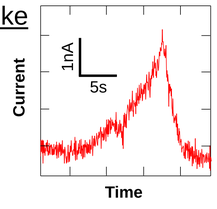


Schematic showing e1) step and e2) spike events depending on cell adsorption to the UME. Presence of cell inhibits oxidation of FeCN. f1) and f2) show representative events from d) and the time-scale difference is apparent

f1) Step

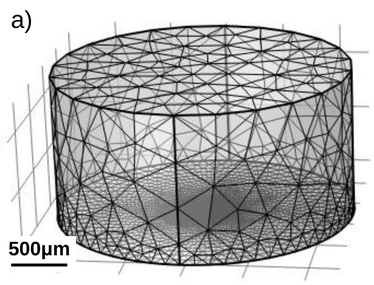


f2) Spike



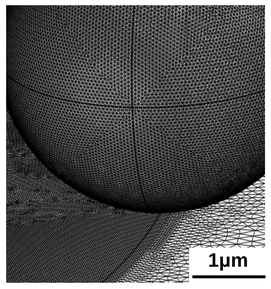
COMSOL Simulations

a)



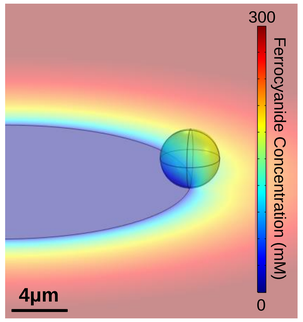
a) Finite element simulation of cylindrical domain with finer elements at the UME for more accurate calculations

b)



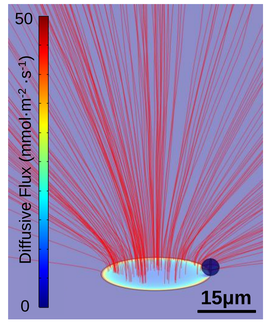
b) Zoomed image of a) showing finest elements at the UME and particle and growing outwards

c)



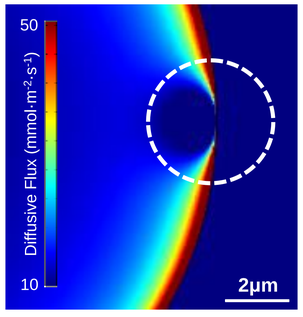
c) When steady-state potential is applied for 100 seconds, FeCN concentration decreases at UME

d)



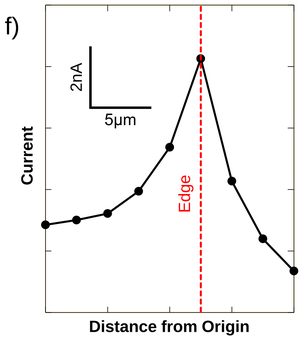
d) Streamline plot showing hemispherical diffusion of FeCN resulting in greater flux at UME edge

e)



e) 2D plot of stationary particle at the UME edge blocking flux of FeCN

f)



f) Particle was manually placed along XY plane to show effect of location on blocking magnitude

Future Work

1. Determine conditions to obtain selectivity of cell adsorption
   - Live cells may be more likely to adsorb based on Fe^{3+} affinity
   - Adsorption may depend on how negatively charged cells are
2. Use particle-tracking module in COMSOL for cell transport
   - Are cells moving mainly by diffusion, migration, or convection
   - Why is there a massive difference in event time-scale
3. Detect direct electrochemical communication with cells
   - Use hydrophilic mediator to probe membrane redox sites
   - Use lipophilic mediator to probe intracellular redox sites
   - Monitor response in real-time as cells are killed

References:

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Acknowledgements

Department of Chemistry, VCU
American Chemical Society
Petroleum Research Fund
Junaid U. Ahmed
Ashley Tubbs
Derek C. Prosser