Scanning Electrochemical Microscopy: Biocatalytic Activity Measurement Via Mercury Electrode

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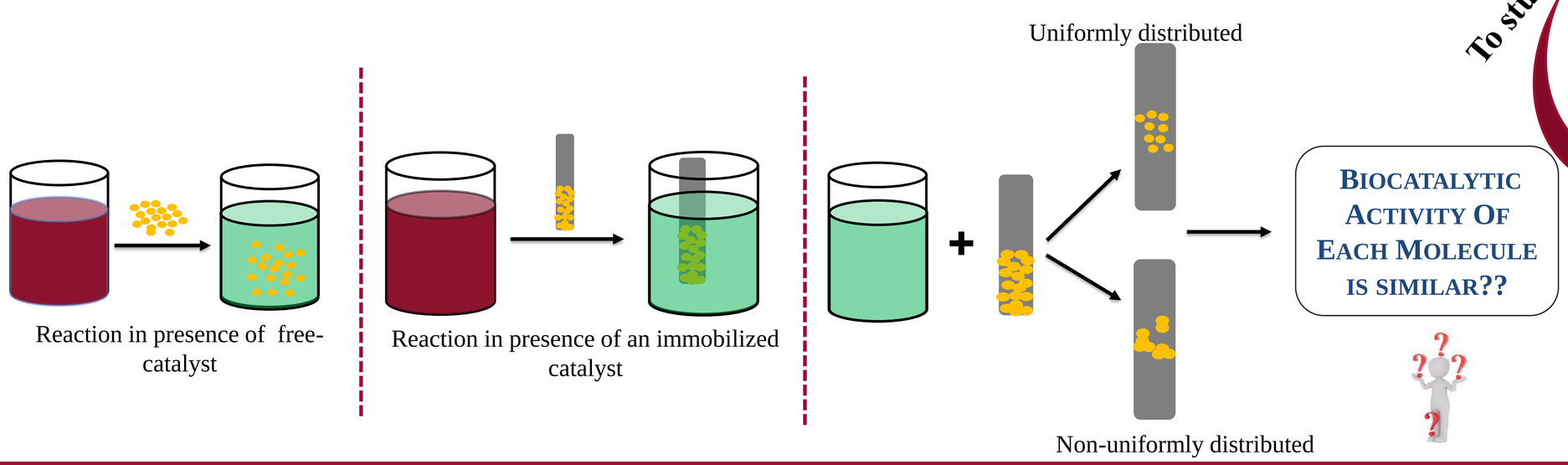
Abstract

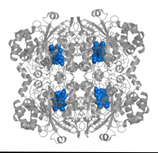
Catalysis plays a central role in almost every reaction because of the advantages that this brings forth. It is one of the most valuable principles of green chemistry. Immobilization of catalyst onto some substrate can reduce one of the major problems of purification of product from the catalyst. If an immobilized catalyst is used, then the chances of recovery as well as reusability increase. The distribution of active sides can impact the activity of catalysts if distributed nonuniformly. Inhomogeneous distribution of active sides results in inefficient catalyst utilization due to competition between adjacent active sides for the same molecules of the substrate of catalytic reaction. So, here, we study the distribution of the biocatalytic activity of the immobilized enzyme (biocatalyst) with scanning electrochemical microscopy (SECM).



To study this…

MATERIAL: CATALASE

Has one of the largest turnover rates known among enzymes



$2H_2O_2 \rightarrow 2H_2O + O_2$

$k_{cat} = 4.4 \times 10^7\ s^{-1}$

Therefore, can be used to study oxygen flux at a single entity level

TECHNIQUE: SECM

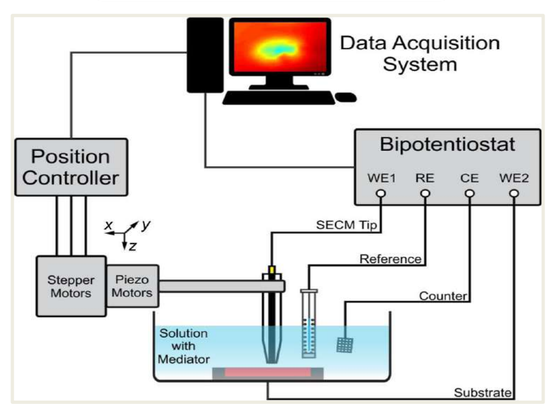
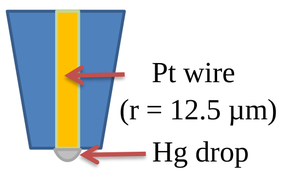


Diagram of SECM instrument

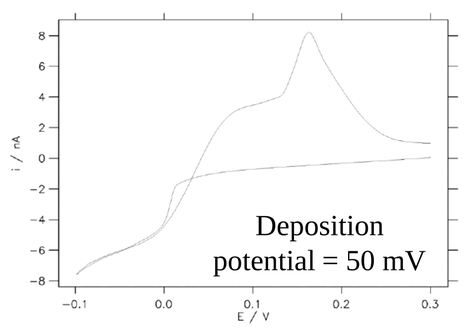
Step 1: Preparation of Hg@Pt microelectrode (ME)

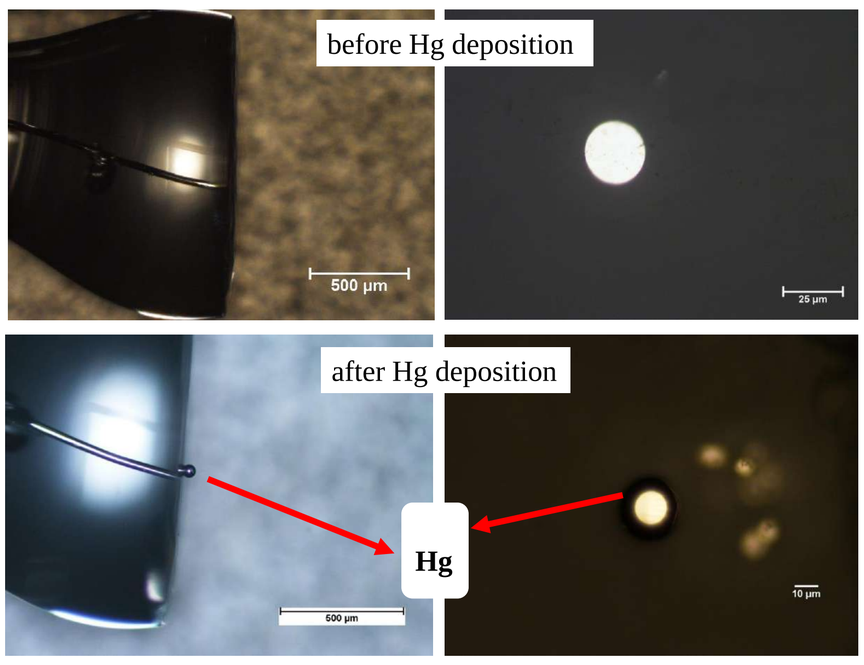


Cyclic Voltammogram for deposition and dissolution of Mercury at Pt ME.

Solution used:

- 1 mM $Hg(NO_3)_2$
- 0.01 M NH_4OAc – HCl (pH = 3)
- 5 mM KSCN



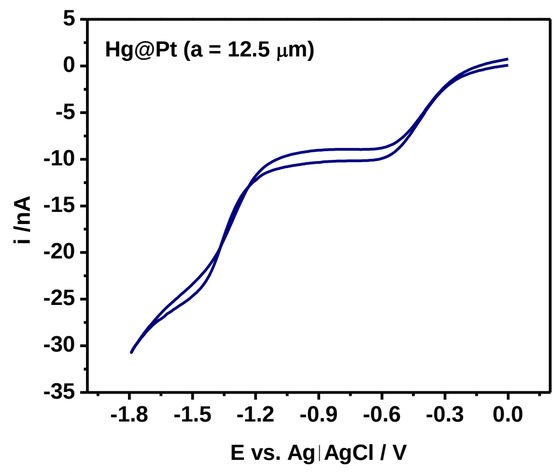


Step 2: Characterization of Hg@Pt ME

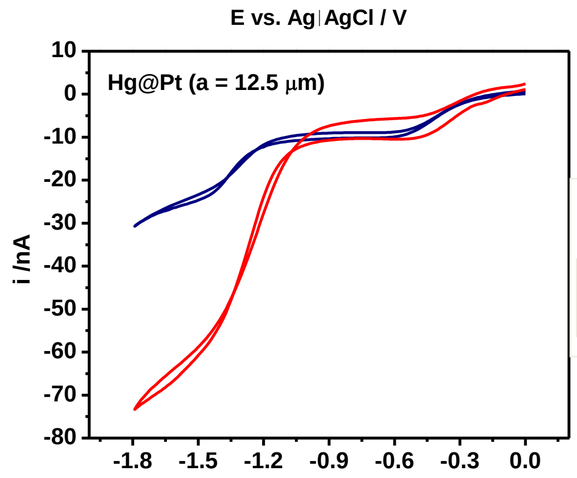
$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$

$H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O$

$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$



Cyclic voltammogram recorded with a 12.5 µm radius Hg@Pt ME in 5 mM Phosphate buffer, pH = 7

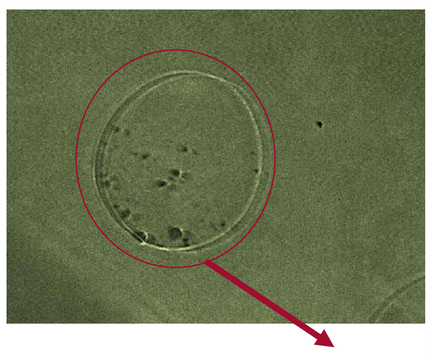


Cyclic voltammograms recorded with a 12.5 µm radius Hg@Pt ME in 5 mM Phosphate buffer, pH = 7 (shown in blue) and 5 mM Phosphate buffer, + 0.5 mM H_2O_2 (shown in red)

Experiments and results

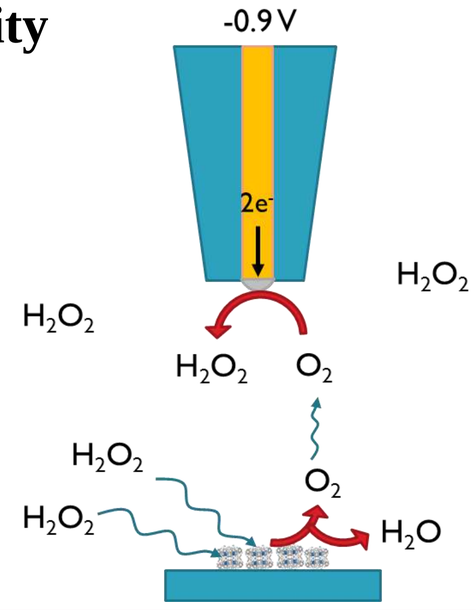
Step 3: Preparation of microspot of catalase

Catalase (beef liver), lyophilized powder, ≥10,000 units/mg protein (Sigma-Aldrich)
1mg of catalase in 1 ml of 50 mM potassium phosphate buffer, pH = 7

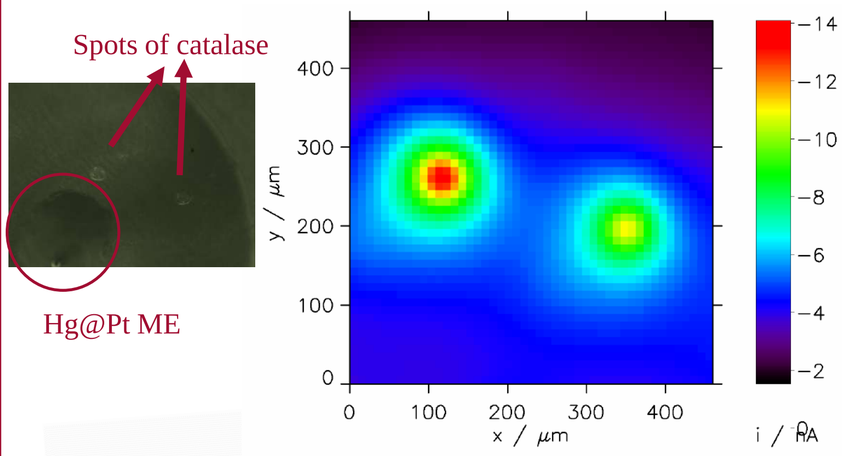


Spot of catalase

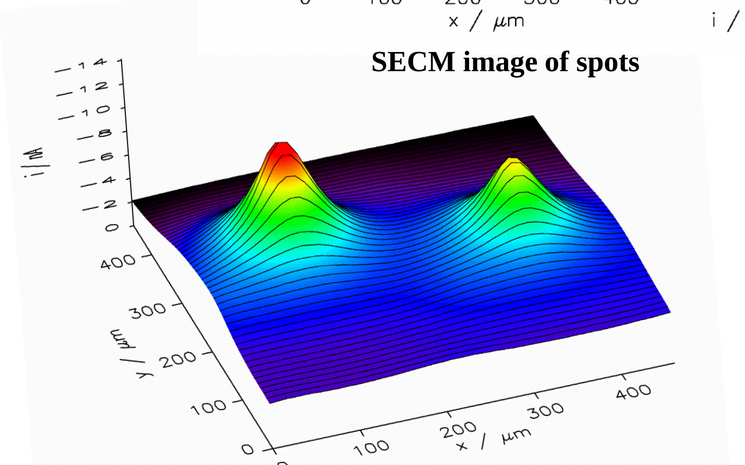
Step 4: SECM imaging of catalase activity



Generation collection mode



SECM image of spots



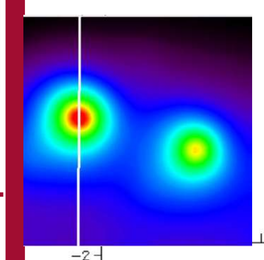
3D representation of SECM image of spots

Step 5: Oxygen flux estimation

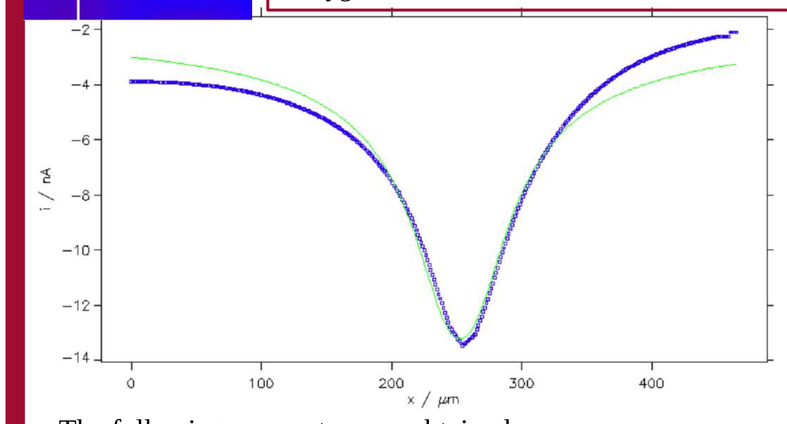
For quantification of the oxygen flux, a cross-section above the centre of the spot has been extracted from a SECM image. The resulting profile has been fitted to equations below:

$$\xi = \frac{2}{\pi} actan \frac{\sqrt{2r_s}}{\left(\sqrt{\Delta x^2 + d^2 - r_s^2\sqrt{(\Delta x^2 + d^2 + r_s^2)^2} + 4d^2 r_s^2}\right)}$$

$$i_T = 4nFD\,C[O_2]r_T\xi$$



n – number of transferred electrons per molecule
F – Faraday constant (96485 C mol^{-1})
D – Diffusion coefficient, 2.0×10^{-5} cm^2/s
$C[O_2]$– bulk concentration of the reagent
r_T – Radius of microelectrode (12.5µm)
ξ - dimensionless factor describing the decrease of dioxygen concentration



The following parameters are obtained::

$2FD[O_2]r_T C[O_2] = -6.6 \times 10^{-9}$ A

$x_0 = 252.75$µm

d (distance b/w substrate & ME) = 23.82 µm

r (radius of substrate scan area) = 19.4 µm

$i_{offset} = -1.7$ nA

$C[O_2] = 1.368$ mM

$\Omega = 2D[O_2]r_T C[O_2]$ **$\Omega = 6.84 \times 10^{-14}$ mol s^{-1}**

Conclusions

- Hg@Pt ME was successfully prepared by electrodeposition from a mercuric salt solution.
- The 2-electron ORR and H_2O_2 RR potentials were well separated at Hg MEs.
- Hg MEs are applicable for catalase activity mapping via generation collection mode.
- Oxygen flux is calculated by analyzing SECM image.

Future steps

- Preparation of small-size Hg@Pt ME and spots of catalase.
- Estimation of Oxygen flux by analyzing SECM image.

Acknowledgment

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References

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[3] A. Roguska, A. Lesniewski, M. Opallo, W. Nogala, *Electrochem. Commun.* 133 (**2021**) 107167.