Electronic Supplementary Material (ESI) for Catalysis Science & Technology


# Supplementary Data

# On the Bio-electrocatalytic Activity of Tyrosinase for Oxygen Reduction Reaction

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## Materials and methods

*Materials:* DSP (bioWORLD, Dublin, OH) was used for immobilizing enzyme on the gold surface. MWNT, 10 nm diameter and 1.5 μm length (Dropsens, Spain) was used as the immobilization support for enzyme on the glassy carbon electrode (GCE). PBSE (Anaspec Inc., Fremont, CA) was used as the heterobifunctional tethering agent for MWNT and enzyme. Dimethyl sulfoxide (DMSO) (VWR, Suwanee, GA) was the solvent used for DSP preparation. Similarly, N,N-dimethyl formamide (DMF) (Thermo Fisher, NJ) was the solvent used for MWNTs and PBSE preparation. Tyrosinase extracted from mushroom (native mushroom/*Agaricus bisporus*, ABD Serotec, UK) was the enzyme used for all experiments. Catechol from Acros Organics was used as mediator in biocatalysis. Industrial grade $O_2$ (Airgas, Atlanta, GA) was used for the experiments. The electrolyte used was 100 ml of 100 mM potassium phosphate buffer (pH 5.8). This phosphate buffer electrolyte was prepared using monobasic and dibasic potassium phosphates (VWR, Suwanee, GA). Distilled and deionized water (18 MΩ conductivity) was used to prepare all the solutions.

*Apparatus:* UV-Visible spectrophotometer (UV-Vis) (Varian Cary 50 Bio, Sparta, NJ) was used to study the activity of tyrosinase. Gold or glassy carbon bare and modified electrodes were used as working electrodes in a three electrode electrochemical cell setup with a platinum wire counter electrode and a silver-silver chloride (Ag/AgCl) reference electrode (CH Instruments Inc, Austin). MWNTs were dispersed in DMF using an ultrasonic homogenizer (Omni International Kennesaw, GA) and XP-Pro ultrasonic cleaner, Sharptek, China. The ORR studies by cyclic voltammetry (CV) were conducted using CHI-920c model potentiostat (CH Instruments Inc, Austin, TX).

*Electrode preparation:* The tyrosinase was immobilized on the gold electrode using DSP, where 0.1 M DSP was casted on the gold surface, after 15 min the surface was washed with deionized water, then 5 mg $mL^{-1}$ of tyrosinase solution was drop casted and incubated for 30 min in the ice bath. The tyrosinase-MWNT composite modified electrode was prepared by modifying the clean bare GCE with 0.13 mg $cm^{-2}$ of MWNT, and then with PBSE as mentioned in our previous work.[S1-S3] Then the tyrosinase solution (5 mg $mL^{-1}$) was drop casted on the modified electrode surface and allowed to immobilize for 30 min in the ice bath.

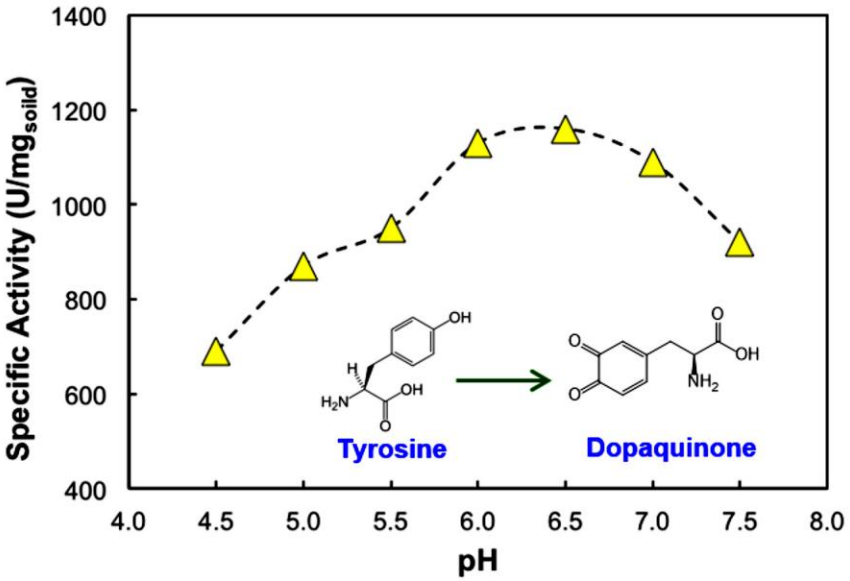


**Figure S1.** Bio-catalytic activity of tyrosinase vs. pH of the solution, determined using UV-Vis spectroscopy by L-tyrosine/Dopaquinone reaction.

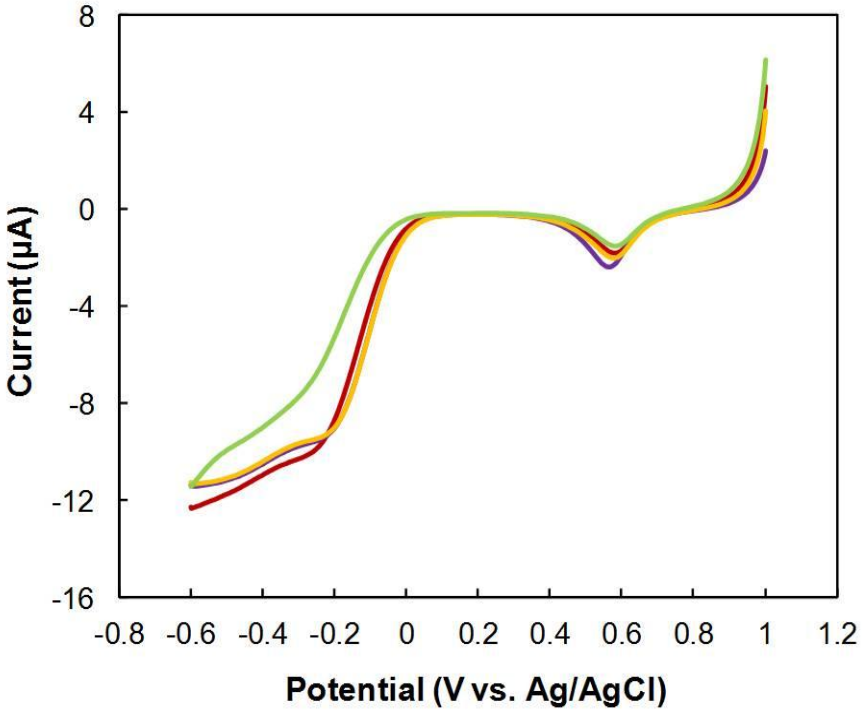


**Figure S2.** Cathodic waves of tyrosinase modified electrode showing the repeatability of $O_2$ reduction reaction. The statistical values such as mean, standard deviation and standard error for the ORR onset potential in these experiments were 0.1 V, 0.01 and 0.007 respectively.

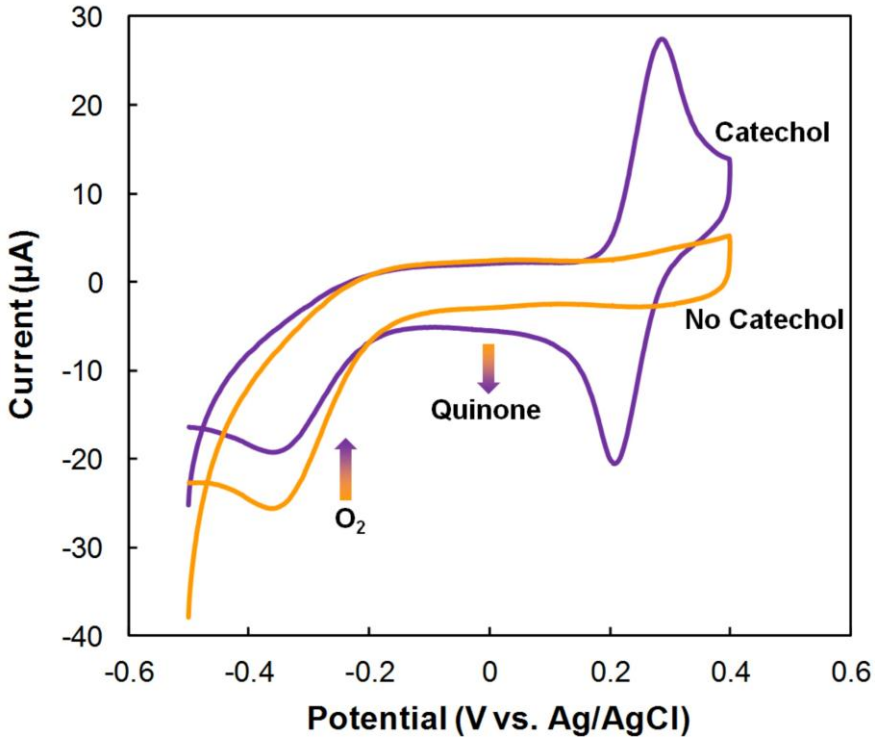


**Figure S3.** Cyclic voltammograms of MWNT-tyrosinase in presence and absence of catechol in $O_2$ saturated phosphate buffer. The potential scanned was -0.5 to 0.4 V vs. Ag/AgCl with the scan rate of 0.02 $Vs^{-1}$. The arrows represent the decrease in ORR current and increase in quinone current respectively.

## References

S1. Ramasamy, R. P.; Luckarift, H. R.; Ivnitski, D. M.; Atanassov, P. B.; Johnson, G. R. *Chem. Comm*. **2010**, *46*, 6045.
S2. Parimi, N. S.; Umasankar, Y.; Atanassov, P.; Ramasamy, R. P. *ACS Catal*. **2012**, *2*, 38.
S3. Lau, C.; Adkins, E.R.; Ramasamy, R.P.; Luckarift, H.R.; Johnson, G.R.; Atanassov, P. *Adv. Energy Mat*. **2012**, *2*, 162.