

Supplementary file

Optimization, Purification and Characterization of Laccase from a new Endophytic *Trichoderma harzianum* AUMC14897 isolated from *Opuntia ficus-indica* and its applications in Dye Decolorization and Wastewater Treatment

Maha M. Salem^{1*}, Tarek M. Mohamed¹, Aya M. Shaban ¹, Yehia A.-G. Mahmoud², Mohammad A. Eid², Nessma A. El-Zawawy²

¹Biochemistry Division, Chemistry Department, Faculty of Science, Tanta University, Tanta, Egypt.

²Botany and Microbiology Department, Faculty of Science, Tanta University, Tanta, Egypt.

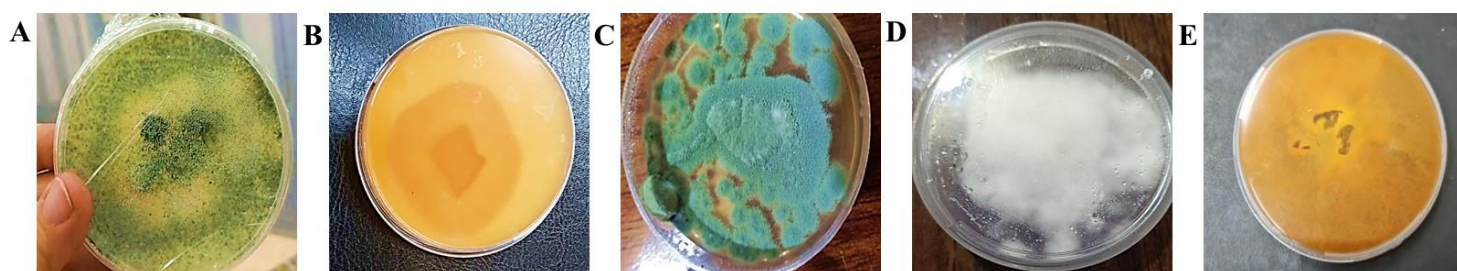


Figure (S1). Fungal endophytes isolated from *O. ficus-indica* and screening of Lacasse activity. A. F-1 strain, B. Positive Lacasse activity of F-1 strain, C. F-2 strain, D. F-3 strain, E. Negative Lacasse activity of F-2 and F-3 strains.

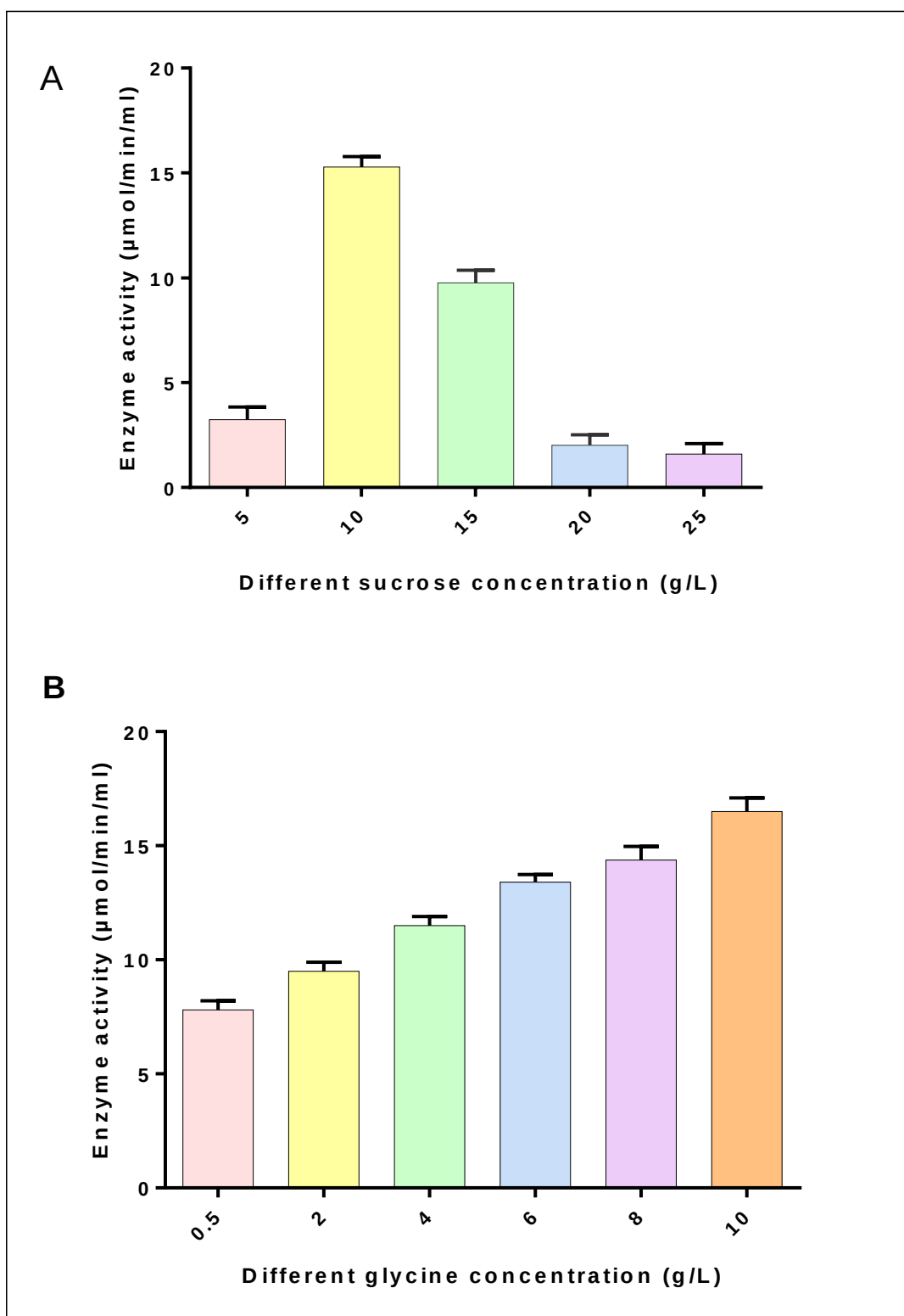


Figure (S2). Effect of nutrients on laccase production by *T. harzianum* AUMC14897 **(A)**. Different sucrose concentration. **(B)**. Different glycine concentration for laccase production from *T.harzianum*

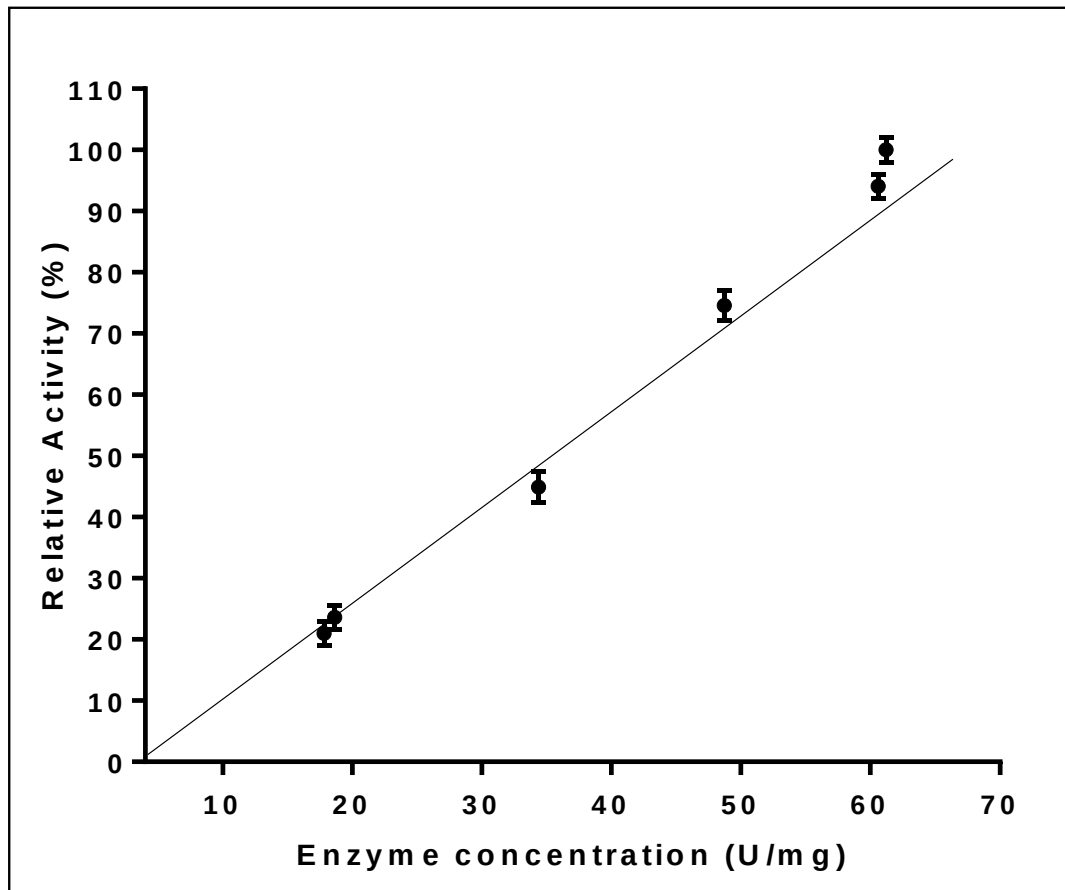
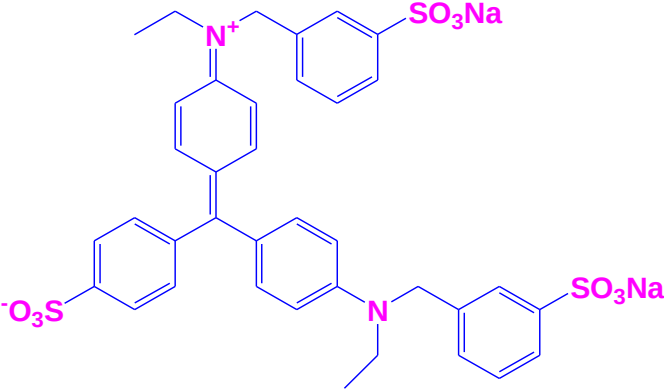
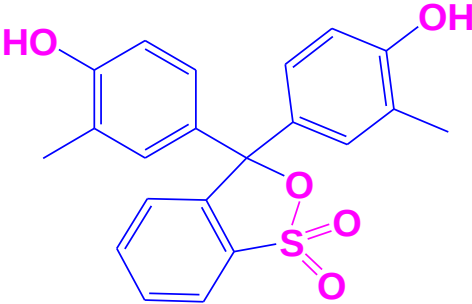
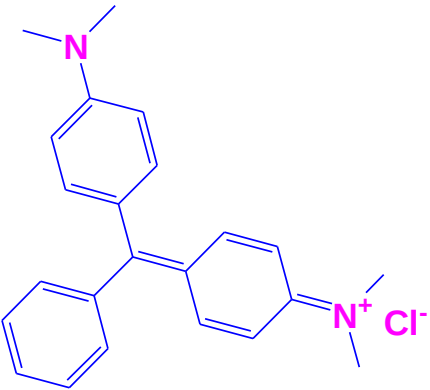
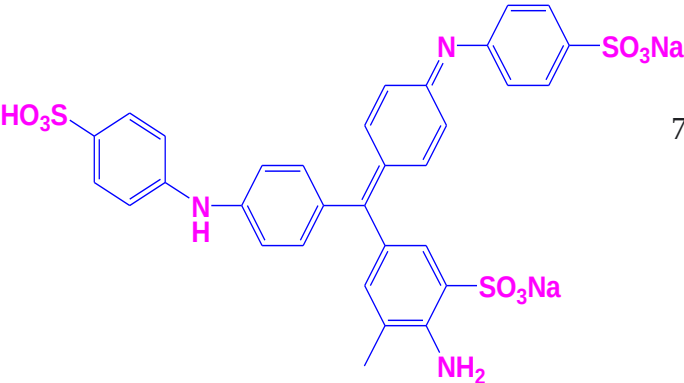


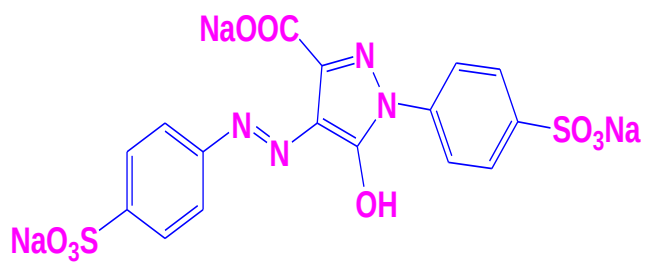
Figure (S3). Effect of different enzyme concentration on purified laccase from *T.harzianum*.

Table S1. Synthetic and environmental real wastewater dyes used in this study

Dyes	Molecular structure	Molecular Weight (g/mol)
Light green SF yellowish ($\epsilon_{\text{max}} = >600$ at 631-634 nm in water)		749.893
Cresol red ($\epsilon_{\text{max}} = 442.01 - 590.16$ nm)		404.41
Malachite green ($\epsilon_{\text{max}} = 617$ nm)		364.91
Aniline blue ($\epsilon_{\text{max}} = 594 - 610$ nm)		737.72

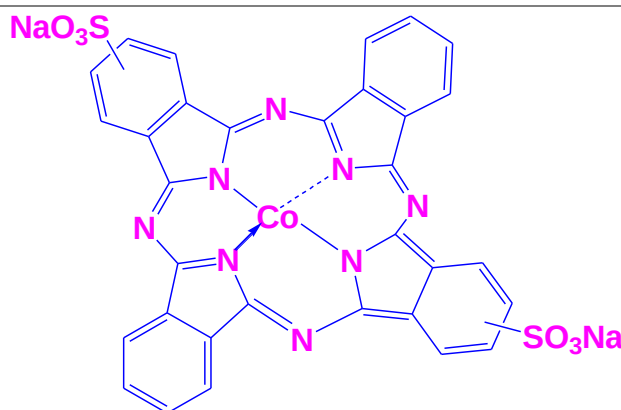
Tartarazine

(ϵ_{max} = 435-500
at 425-429 nm in
water)



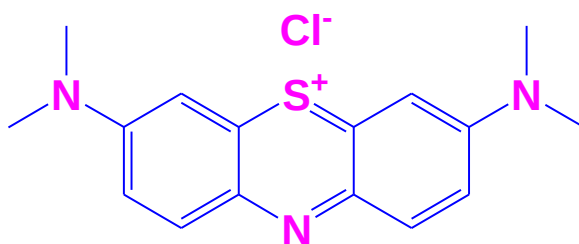
534.3

**Fast
turquoise
blue**
(ϵ_{max} = 550 nm)



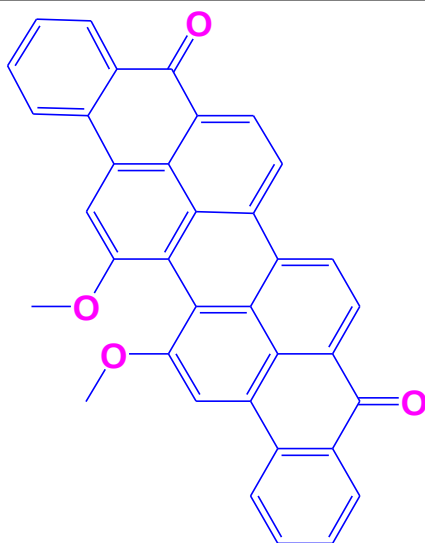
780.17

**Methylene
blue**
(ϵ_{max} = 665 nm)



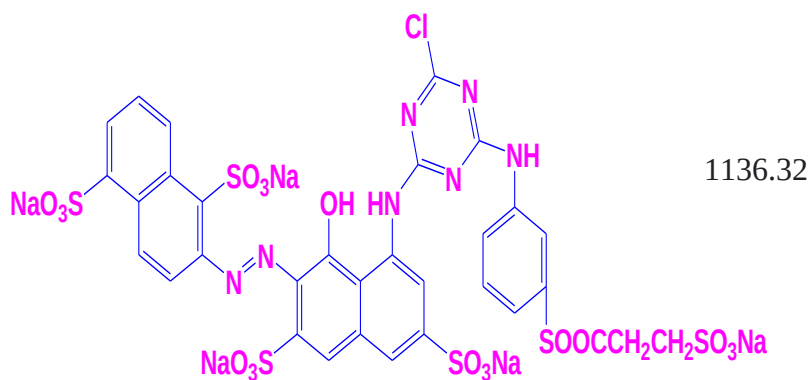
319.85

**Novatic
green XBN**
(ϵ_{max} = 527 nm)



516.64

Red 4BL
(ϵ_{max} = 655 nm)



Reactive T.
Blue G
(ϵ_{max} = 555 nm)

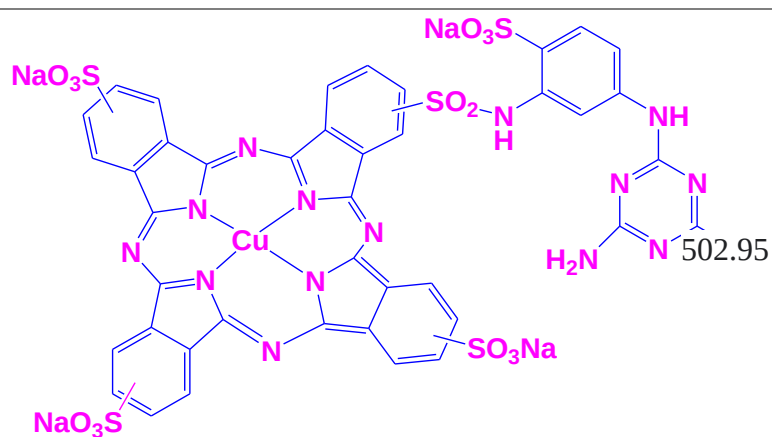


Table S1 Continued

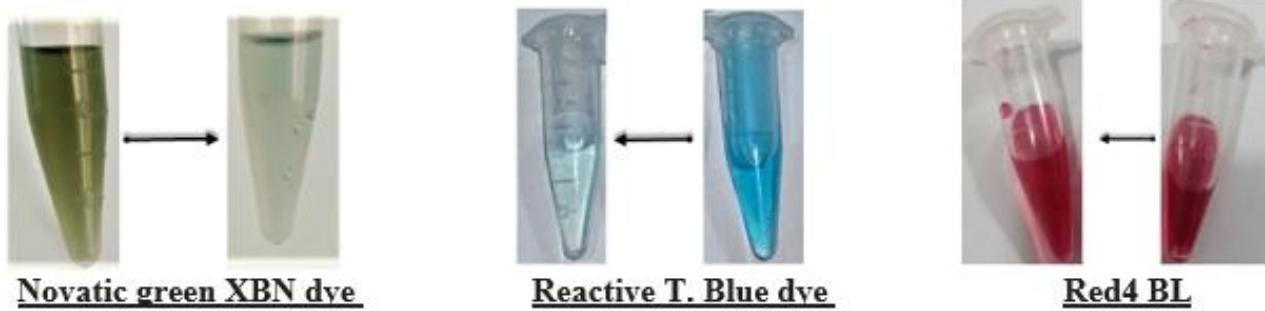


Figure S4: The images for real wastewater dye samples before and after 24h treatment with laccase

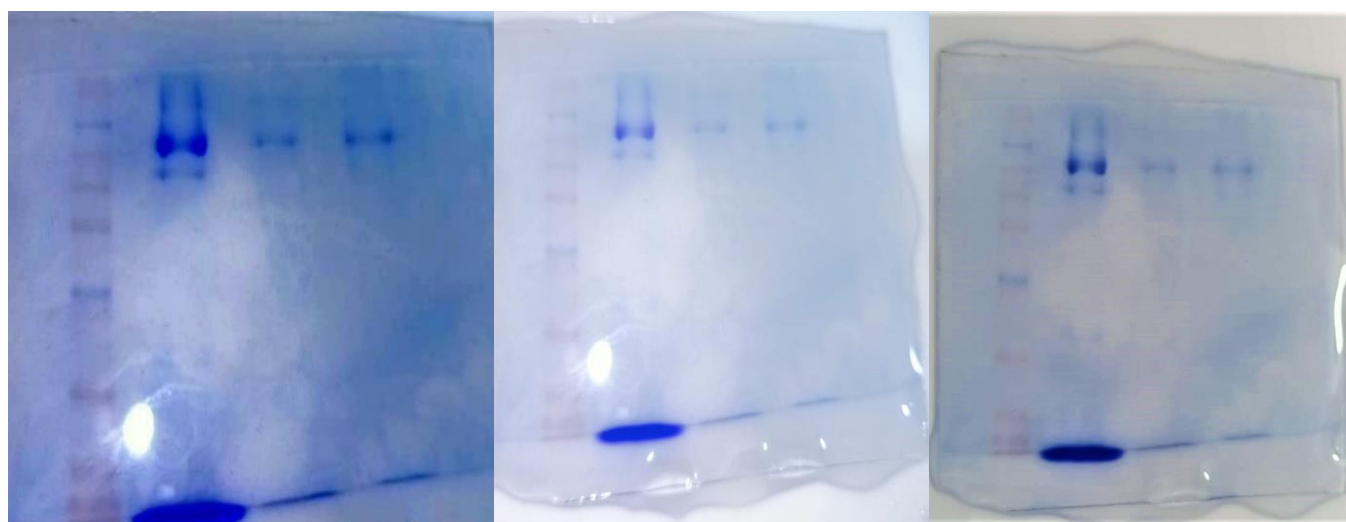


Figure S5: The raw images SDSPAGE for laccase different purification steps

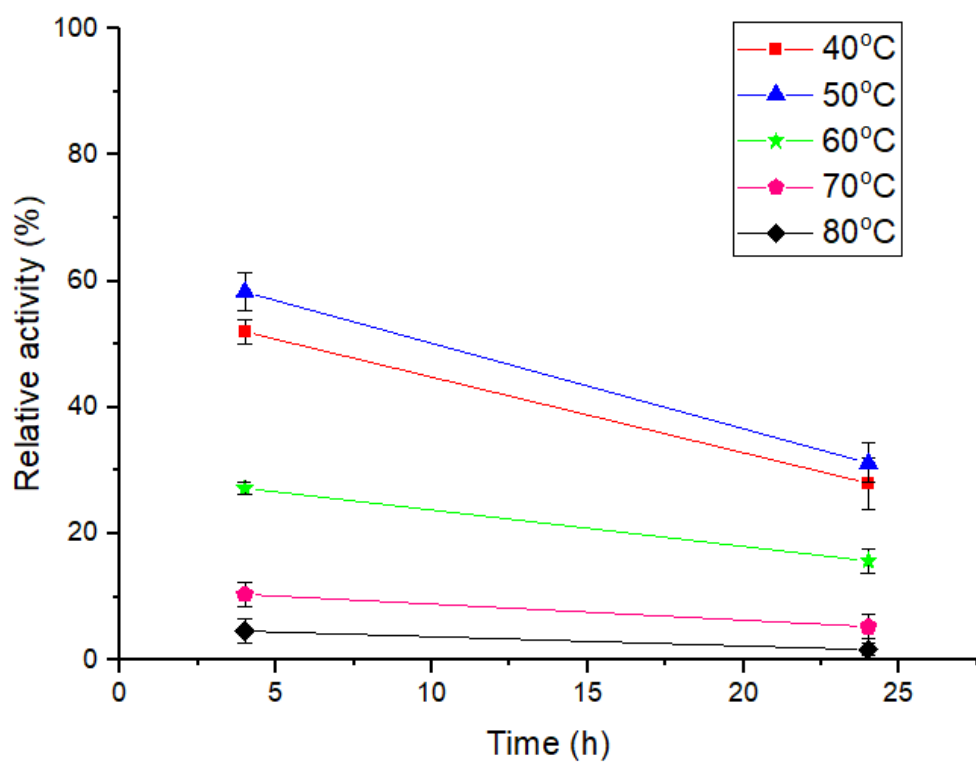
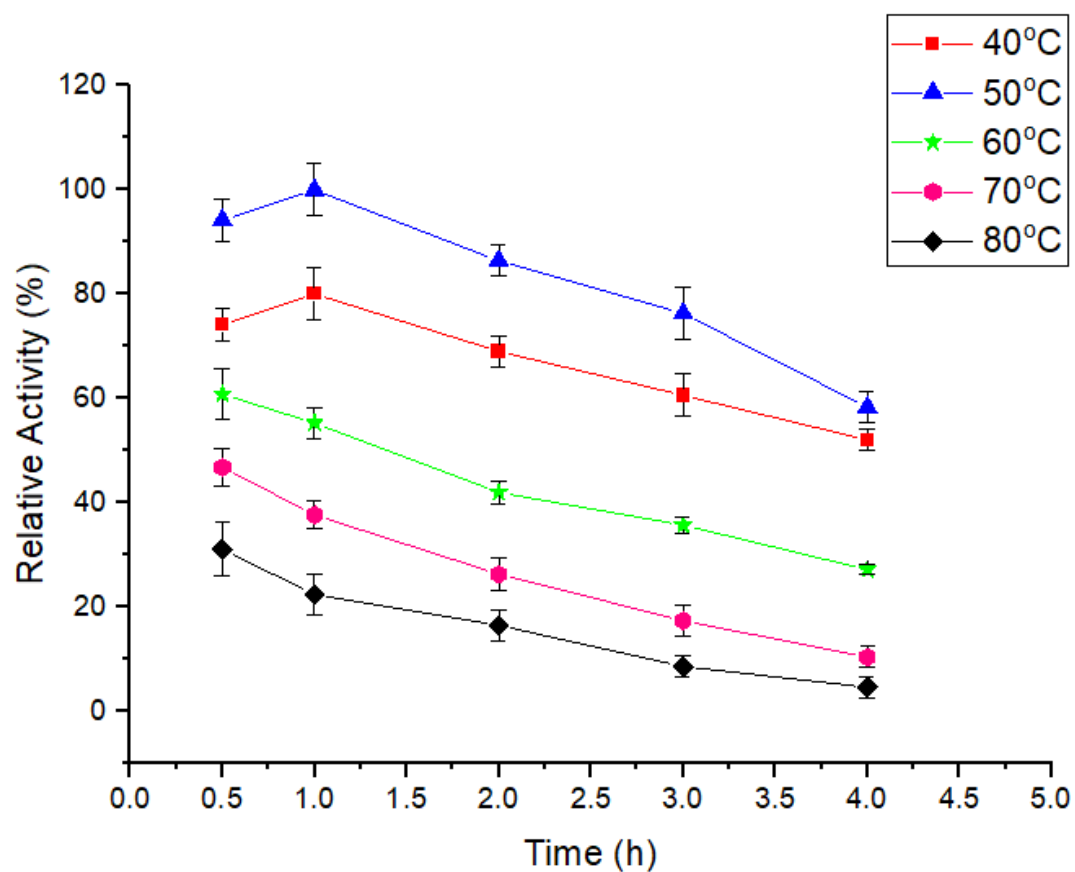


Figure S6: The analysis emphasizes the initial rapid changes in relative enzyme activity during the first 5 hours, followed by a plateau over the next 20 hours.

Mechanisms of laccase for dye decolorization used in our study

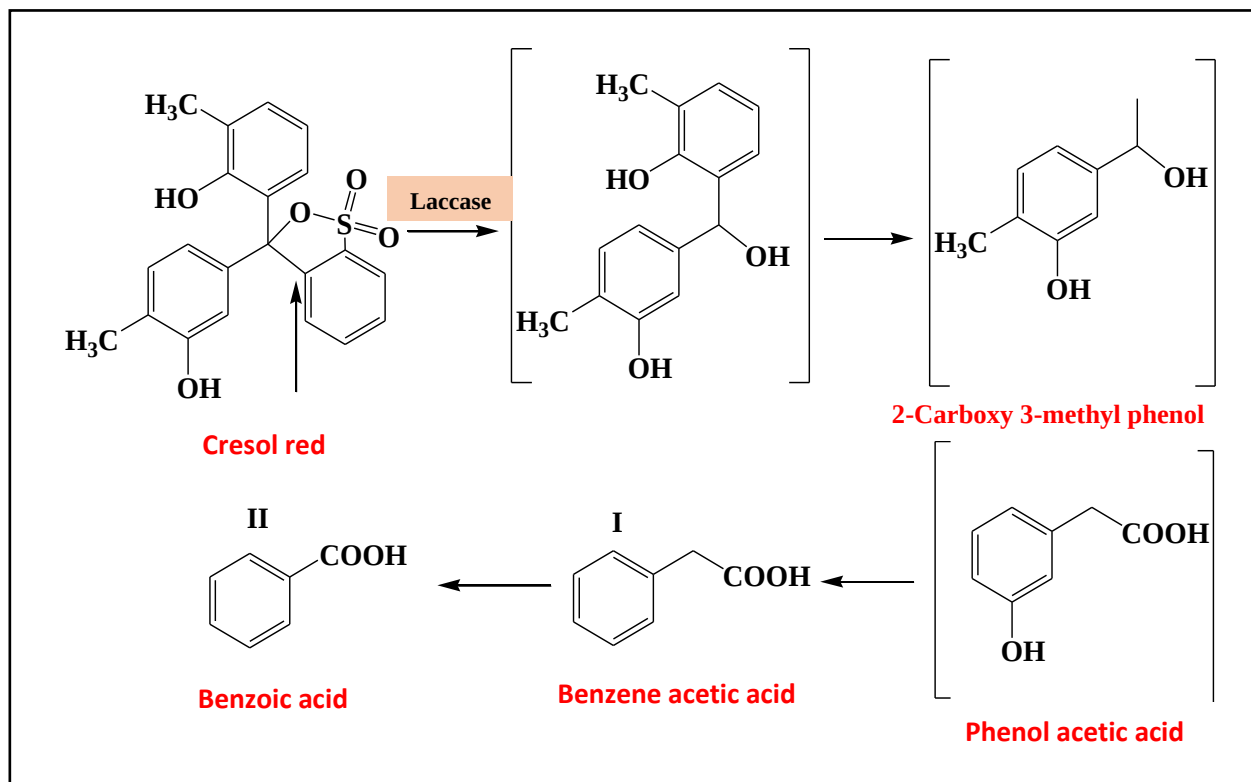


Figure S7. Degradation of cresol red by laccase

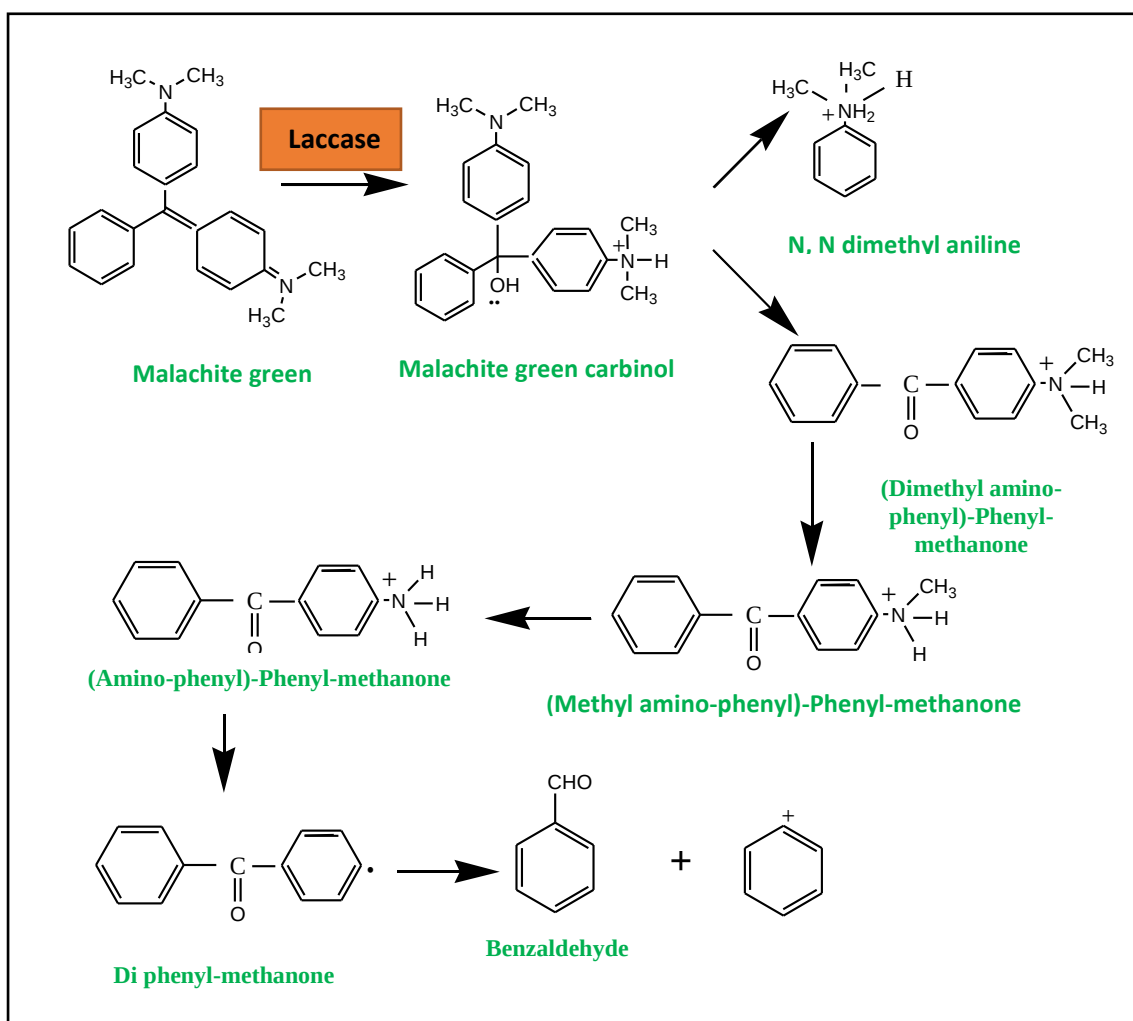


Figure S8. Degradation of malachite green dye by laccase

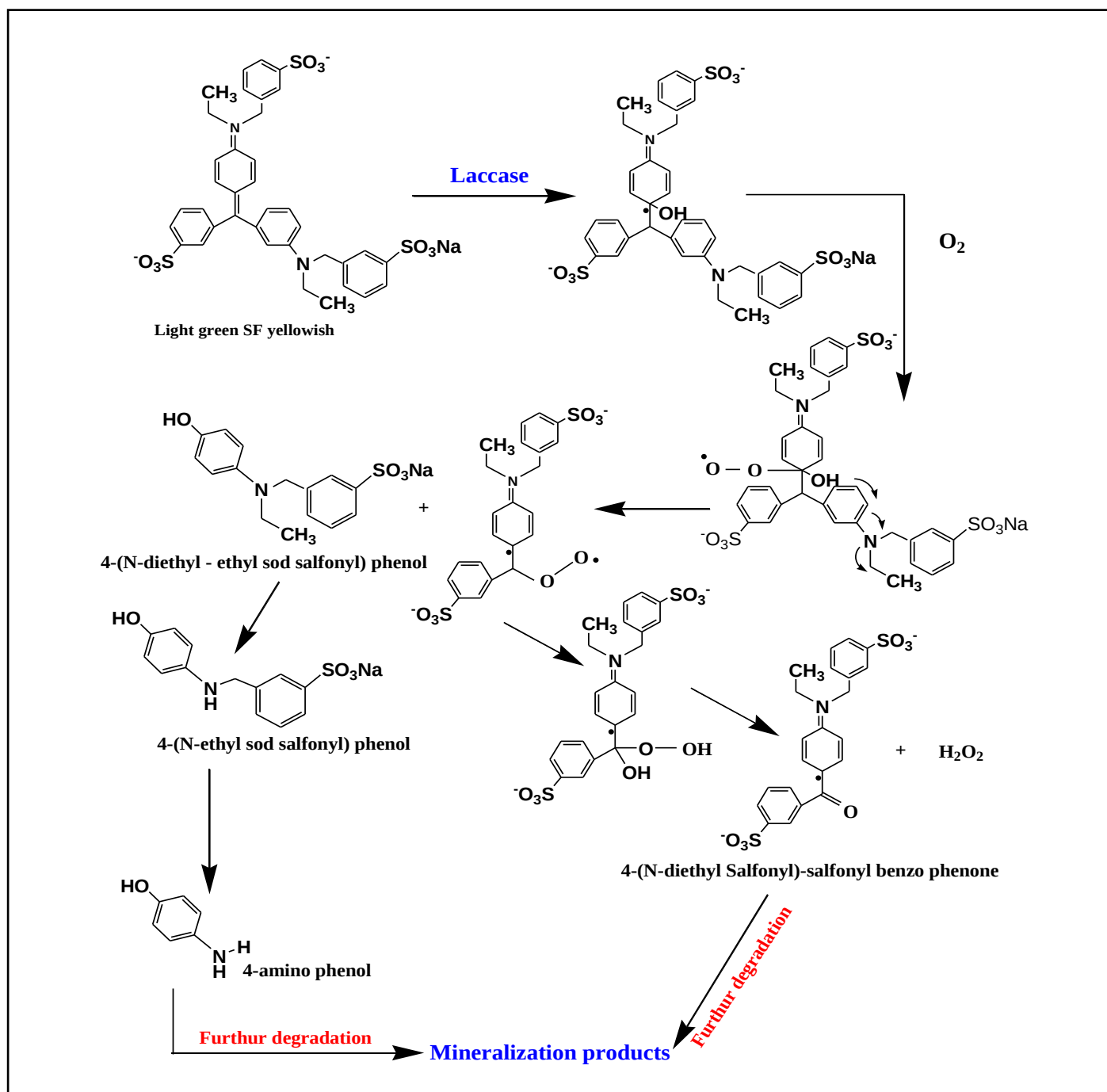


Figure S9. Degradation of light green SF yellowish dye by laccase

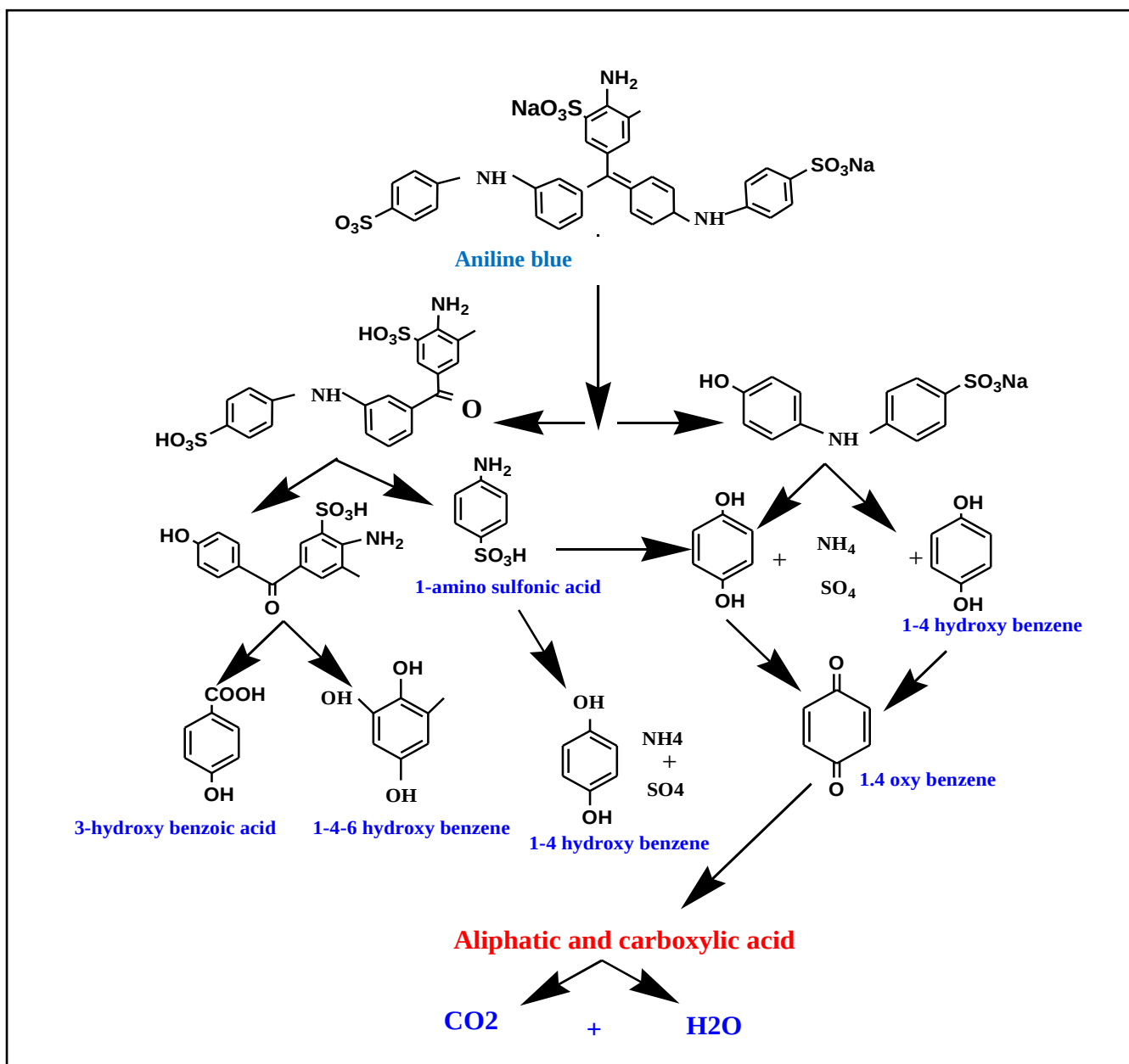


Figure S10. Degradation of aniline blue dye by laccase

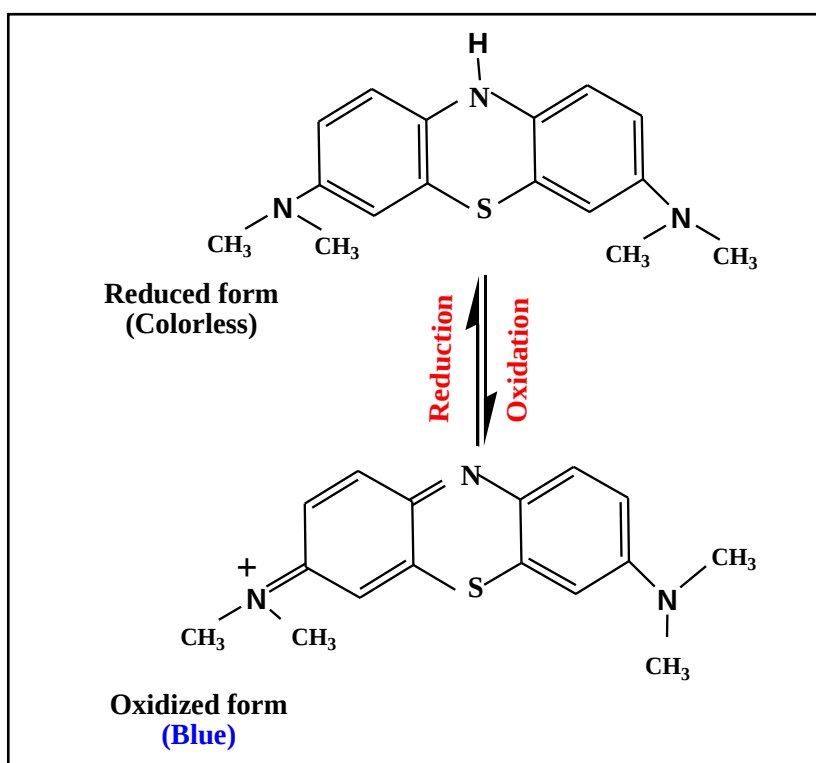


Figure S11. Oxidized and reduced form of methylene blue

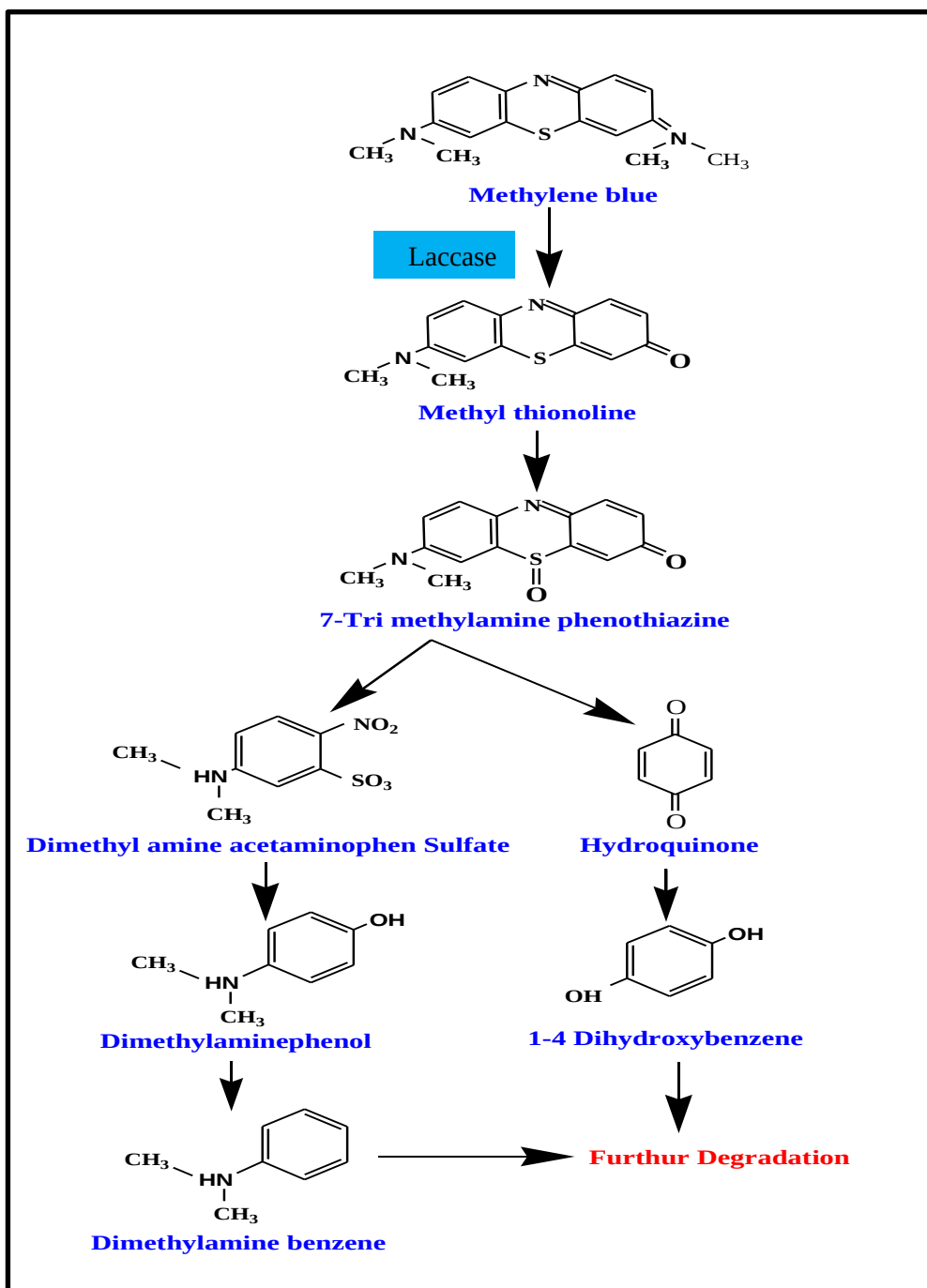


Figure S12. Degradation of methylene blue dye by laccase

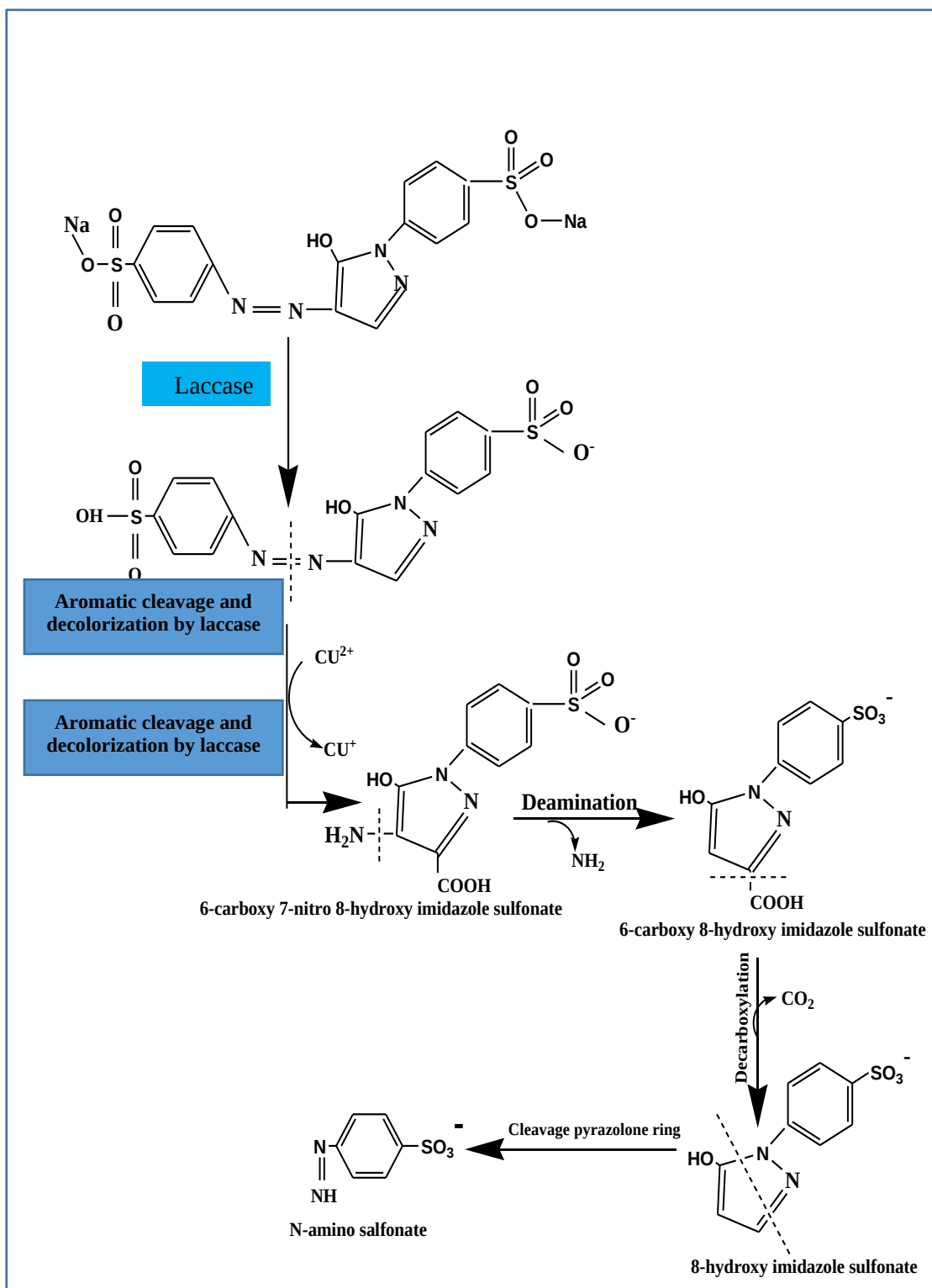


Figure S13: Degradation of thiazine azo dye by laccase

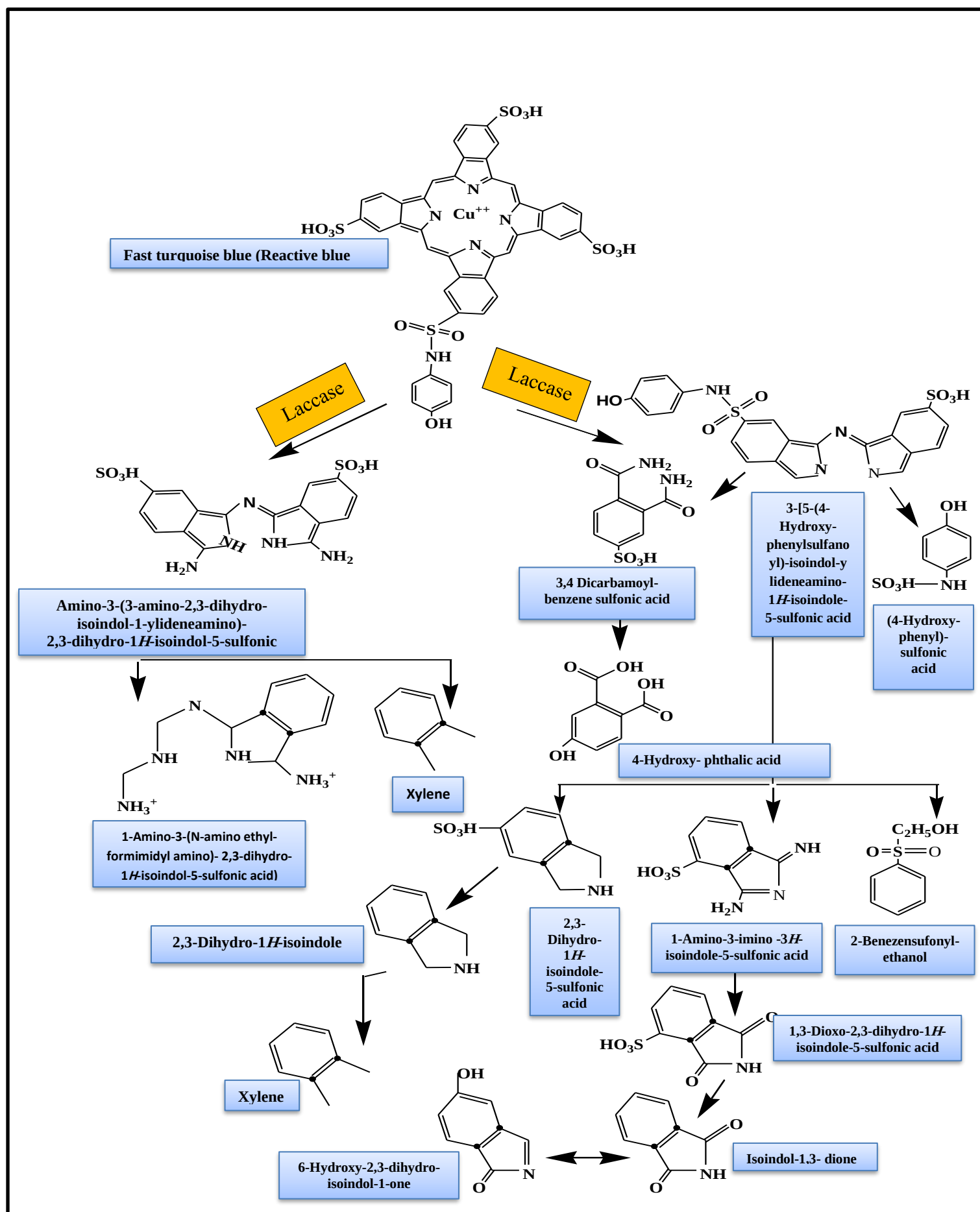


Figure S15. Degradation pathway of fast turquoise blue dye by laccase