

## Biodegradation of acid dyes by an immobilized laccase: an ecotoxicological approach

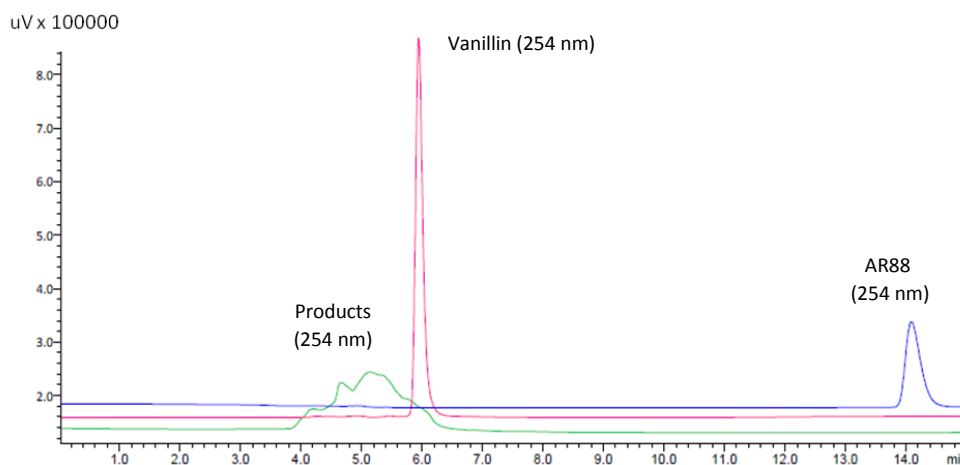
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### SUPPLEMENTARY DATA

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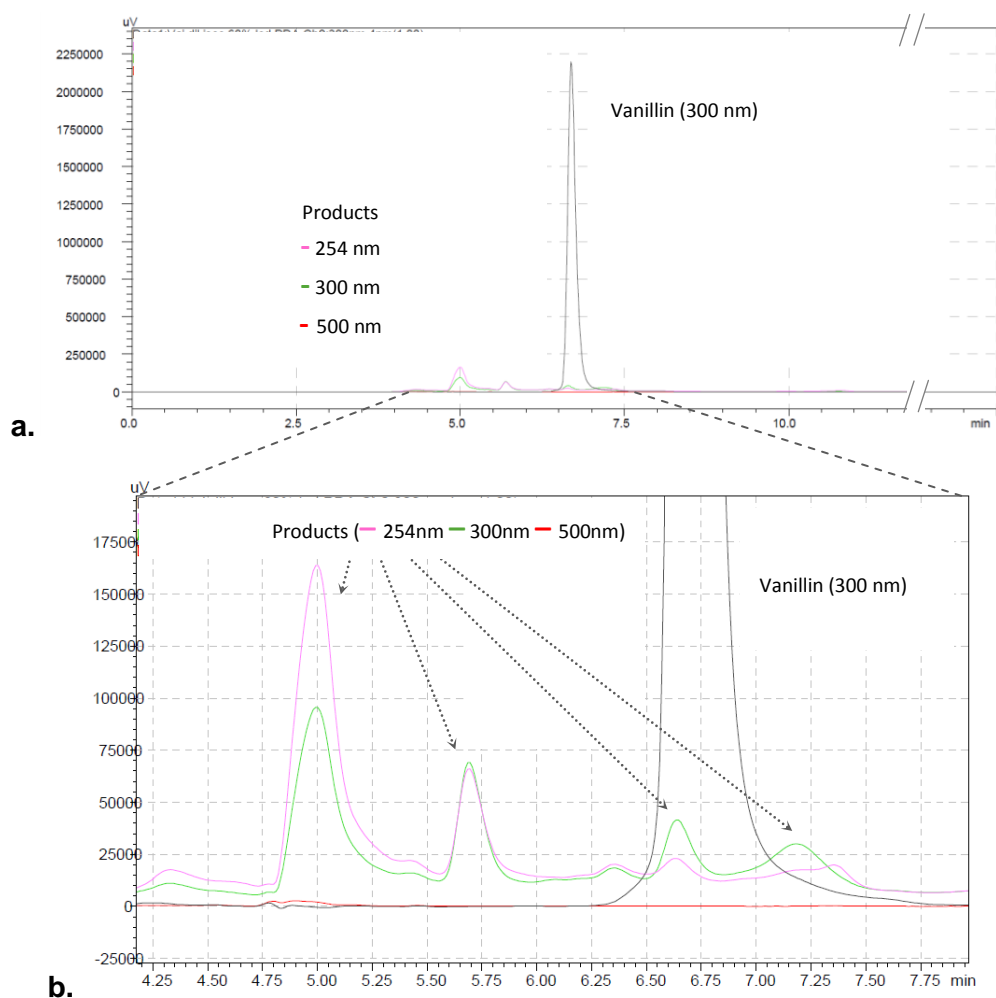
HPLC analysis of AR88 (A) and AB172 (B) degradation by the laccase-mediator system. Analytical conditions: Zorbax Eclipse XDB-C18 column (Agilent Technologies, Santa Clara, CA, USA) of 250 mm, 4.6 mm i.d., and 5  $\mu$ m particle size, with a guard cartridge Ultra-C18 of 10 mm and 4 mm i.d; oven temperature 30 °C, flow rate 0.5 mL/min, injection volume 20  $\mu$ L. Solvent A: 30 mM ammonium acetate (pH 4.6); solvent B: methanol.

#### (A) Analysis of Acid Red 88 degradation by HPLC



**Figure 1.**

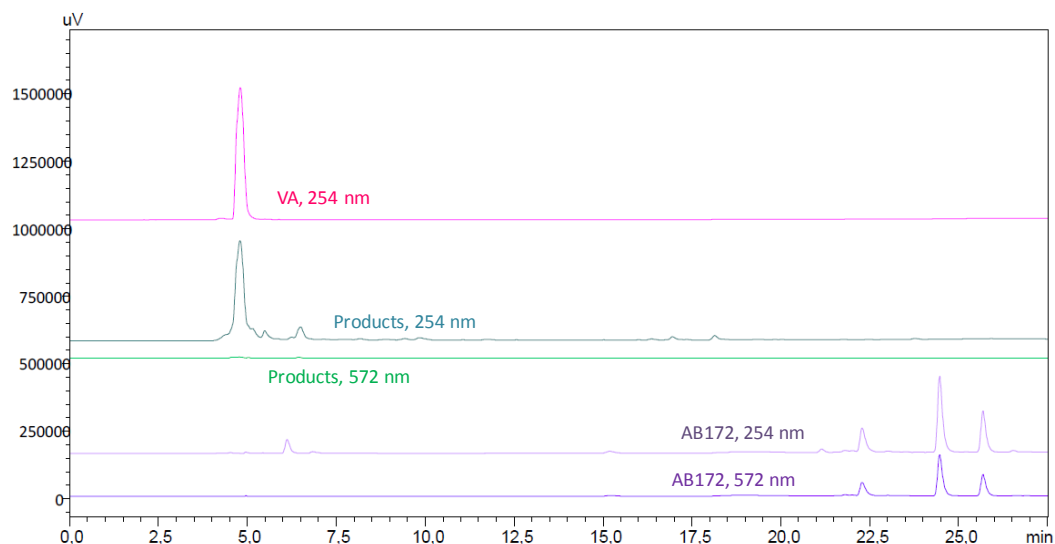
Chromatograms overlapped of AR88, vanillin and degradation products at 254 nm. Program used: 0 – 15 min, 70 - 90 % B; 15–16 min, return to initial conditions.  $t_R$  AR88 = 14.3 min;  $t_R$  vanillin = 6 min;  $t_R$  degradation products (not resolved) = 4 - 6.2 min. The wavelength 254nm was selected for the UV region of the spectrum where all the compounds absorb.



**Figure 2.**

**a.** Chromatograms overlapped of vanillin (at 300 nm) and degradation products of AR88 (at 254, 300 and 500 nm). **b.** Enlargement of the chromatogram between 4 and 8 minutes. Isocratic elution: 20 min, 60 % B.  $t_R$  vanillin = 6.7 min;  $t_R$  main degradation products = 5, 5.7, 6.65 and 7.2 min. The wavelengths 300nm and 500nm correspond to peaks of maximum absorption for vanillin and AR88 respectively.

## (B) Analysis of Acid Black 172 degradation by HPLC



**Figure 3.** Chromatograms of AB172 and degradation products at 254 and 572 nm, and violuric acid (VA) at 254 nm. Gradient elution program: 0–15 min, 40–80% B; 15–30 min, 80% B, 30–31 min, return to initial conditions.  $t_R$  violuric acid = 5 min;  $t_R$  AB172 isomers = 22.3 min (A), 24.5 min (B) and 25.7 min (C). The wavelength 572nm corresponds to the maximum absorption for AB172 in visible spectrum, and 254nm was selected for the UV region of spectrum.