

# Development of a Bioremediation Process Based on Immobilized Laccases

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## Aim of the Work

A research program has been recently started at the Università di Napoli Federico II aiming at the biodegradation of dyes by means of laccases from *Pleurotus ostreatus*. The program subtasks are: i) setting up protocols for the immobilization of laccases on selected granular materials; ii) assessment of activity and stability of immobilized laccases under surrogate and real process conditions.

## Materials and Methods

- Microorganism: *Pleurotus ostreatus* (Jacq.:Fr.) Kummer (type Florida).
- Enzyme carrier [1]: acrylic epoxy-activated beads
  - i) EUPERGIT C 250L<sup>®</sup> bulk density= 0.3 kg/m<sup>3</sup>, particle size 180 μm, pore size 100 nm, oxirane content ~ 0.3 mmol/ g
  - ii) EUPERGIT C<sup>®</sup> bulk density 0.3 kg/m<sup>3</sup>, particle size 100 μm, pore size 2 nm, oxirane content ~ 0.6 mmol/ g (Sigma).
- Dye: anthraquinonic reactive dye, Remazol Brilliant Blue R λ<sub>max</sub> = 592 nm (RBBR, Sigma).
- Laccase mixtures were recovered from *P. ostreatus* culture broths and assayed according to the procedure described in [2].

## Result 1

### Laccase immobilization.

Immobilization protocol was optimized studying influence of ratio between liquid phase laccase activity and mass of carrier, pH, ionic strength and reaction time. Residual epoxy groups on carrier surface were saturated in a 4 g/L Bovine Serum Albumine (BSA, Sigma) solution, samples were stored at 4°C in light buffer.

- **Immobilization yield = (immobilized activity)/(initial activity) was as high as 7%** for experiments carried out at: initial activity 85 IU/g<sub>dry beads</sub>, total proteins content 2±5 mg/g<sub>dry beads</sub>. **Activity recovered in supernatant was about 60%.**

## Result 2

- **A bench scale facility has been specifically developed to characterize biocatalyst performances.** The heart of the experimental setup is a tubular fixed bed reactor loaded with laccases immobilized on EUPERGIT<sup>®</sup> C 250L. Enzyme assaying was accomplished according to configuration **Fig. 1A**, i.e. by recycling the product stream. Concentration of products from oxidation of ABTS was time-resolved by means of an on-line spectrophotometer. Dye (RBBR) degradation kinetic was determined according to configuration **Fig. 1B**, i.e. with continuous feeding of dye solution and continuous monitoring of RBBR optical absorbance at the reactor outlet.

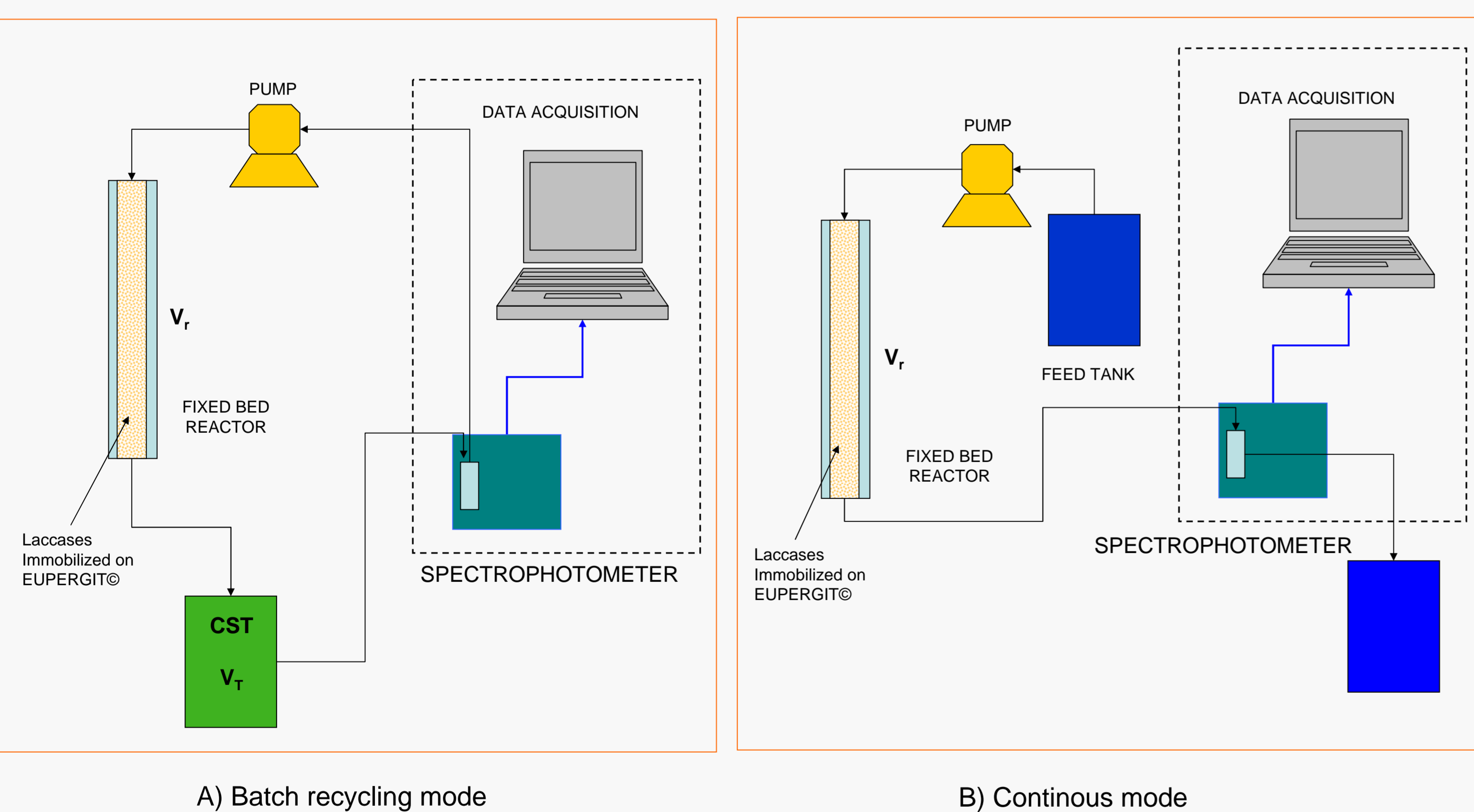


Fig.1: Experimental apparatus

## Modelling

- **Immobilized laccase assay.** The substrate mass balance equation applied to recycle reactor in Fig. 1A, under the hypothesis that the fixed bed reactor is operated as differential with respect to the substrate, is

$$-\frac{ds}{dt} = \frac{V_r}{V_T} \cdot r(e_0, s, p_i, \dots)$$

s substrate concentration  
e<sub>0</sub> enzyme concentration  
p<sub>i</sub> products concentration  
r reaction rate

Eq. 1

The intrinsic kinetic may be quantitatively assessed provided that conversion rate is not affected by interphase mass transfer. The Damköhler number (Da), expressing the ratio between mass transfer in the boundary layer and the reaction rate, is defined by Eq. 2:

$$Da = \frac{r \cdot a}{k_s \cdot s_0}$$

a catalyst surface per unit volume of particles bed  
k<sub>s</sub> external mass transfer coefficient

Eq. 2

k<sub>s</sub> was assessed using correlation for the Sherwood number as a function of Schmidt and Reynolds numbers for fixed bed of spherical particles in the laminar flow regime [3].

- **RBBR degradation kinetic.** Mass balance on RBBR at steady state assuming plug flow behaviour (Fig.1B) and Michaelis-Menten kinetic for c ~ K<sub>m</sub> yields

$$\frac{(1-\varepsilon)}{\varepsilon} \cdot \tau = \frac{v_{max}}{K_m} \cdot L \ln \left( \frac{c_0}{c} \right)$$

c dye concentration  
τ space-time  
c<sup>0</sup> dye concentration in feeding stream  
ε bed void degree

Eq. 3

## Result 3

- **Assessment of enzyme activity and evaluation of immobilization yield.**

**Figure 2** shows the time-resolved optical absorbance at 420 nm. The slope of the curve was analysed according to **Eq. 1** to evaluate the immobilized enzyme activity and the immobilization yield. Diffusional limitations could be ruled out since **Da ~ 10<sup>-3</sup>**. **Therefore, the measured apparent reaction rate reflects the intrinsic reaction kinetic.**

- **Preliminary assessment of RBBR conversion rate.**

Adsorption of RBBR on inert EUPERGIT<sup>®</sup> surface was investigated. Saturation of the solid carrier corresponds to a concentration of 10 mg dye/g<sub>dry beads</sub> (T = 23±0.5°C, Na Acetate 20 mM pH = 4.5). Equilibrium was approached in about one hour (data not shown) under the test conditions.

**Figure 3** reports the concentration of RBBR recorded at the reactor outlet during continuous operation of the reactor. The early stage reflects transient adsorption of dye on the biocatalyst. Multiple regimes were achieved by decreasing stepwise the feeding rate (i.e. the space-time τ). Data were analyzed according to the integral method (**Eq. 3**) to obtain the maximum reaction rate of a Michaelis-Menten kinetic: v<sub>max</sub> 3·10<sup>-4</sup> mg/(min IU); (Hyp. K<sub>M</sub> = 33 mg/L) (**Fig. 4**).

## Future assessments.

**Dye conversion kinetic will be characterized over a wide range of operating conditions (dyes, buffer) using laccases immobilized on several granular supports (next step: activated perlite). Enzyme deactivation rate will also be assessed.**

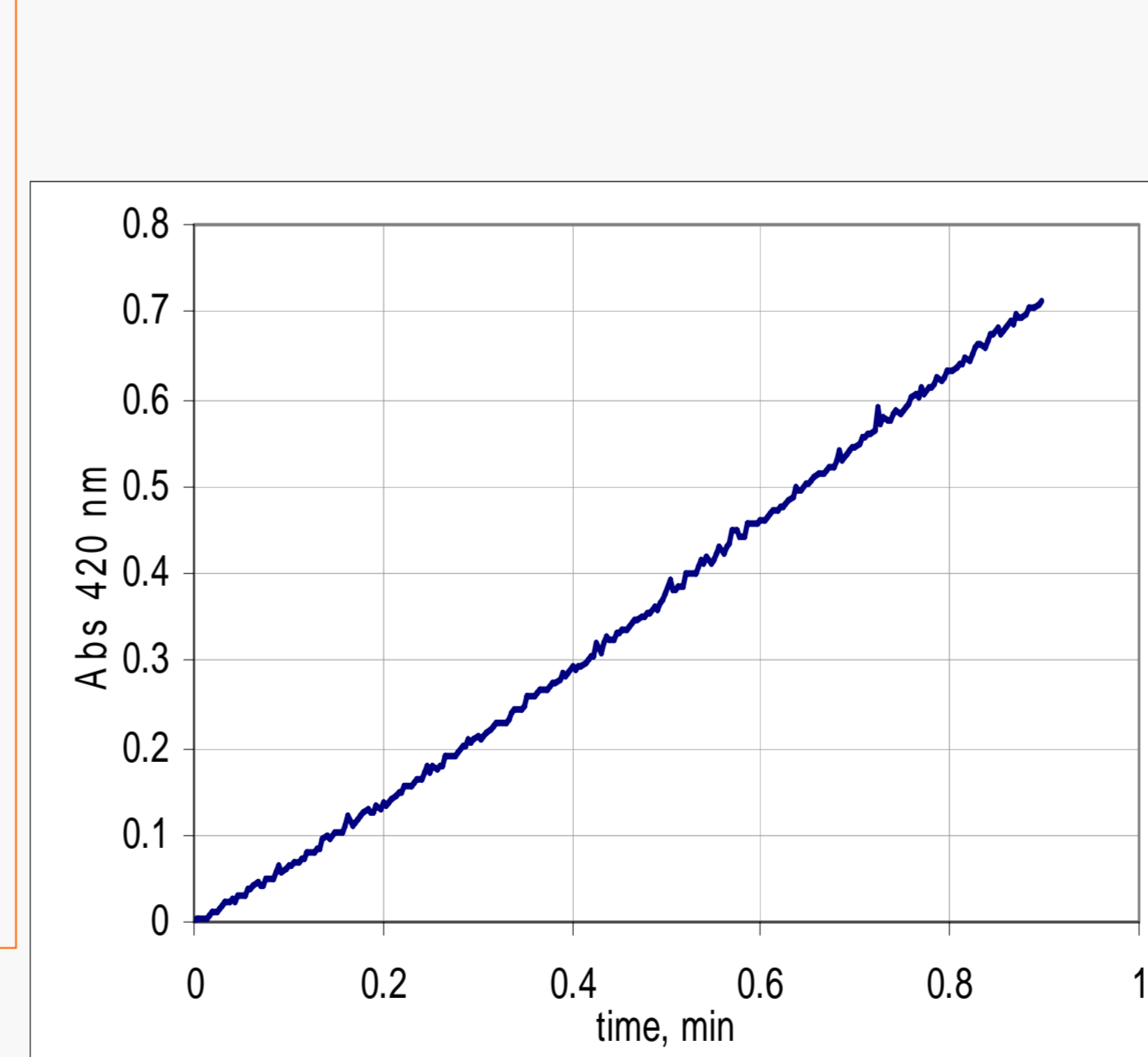


Fig. 2. Immobilized laccases activity assay.

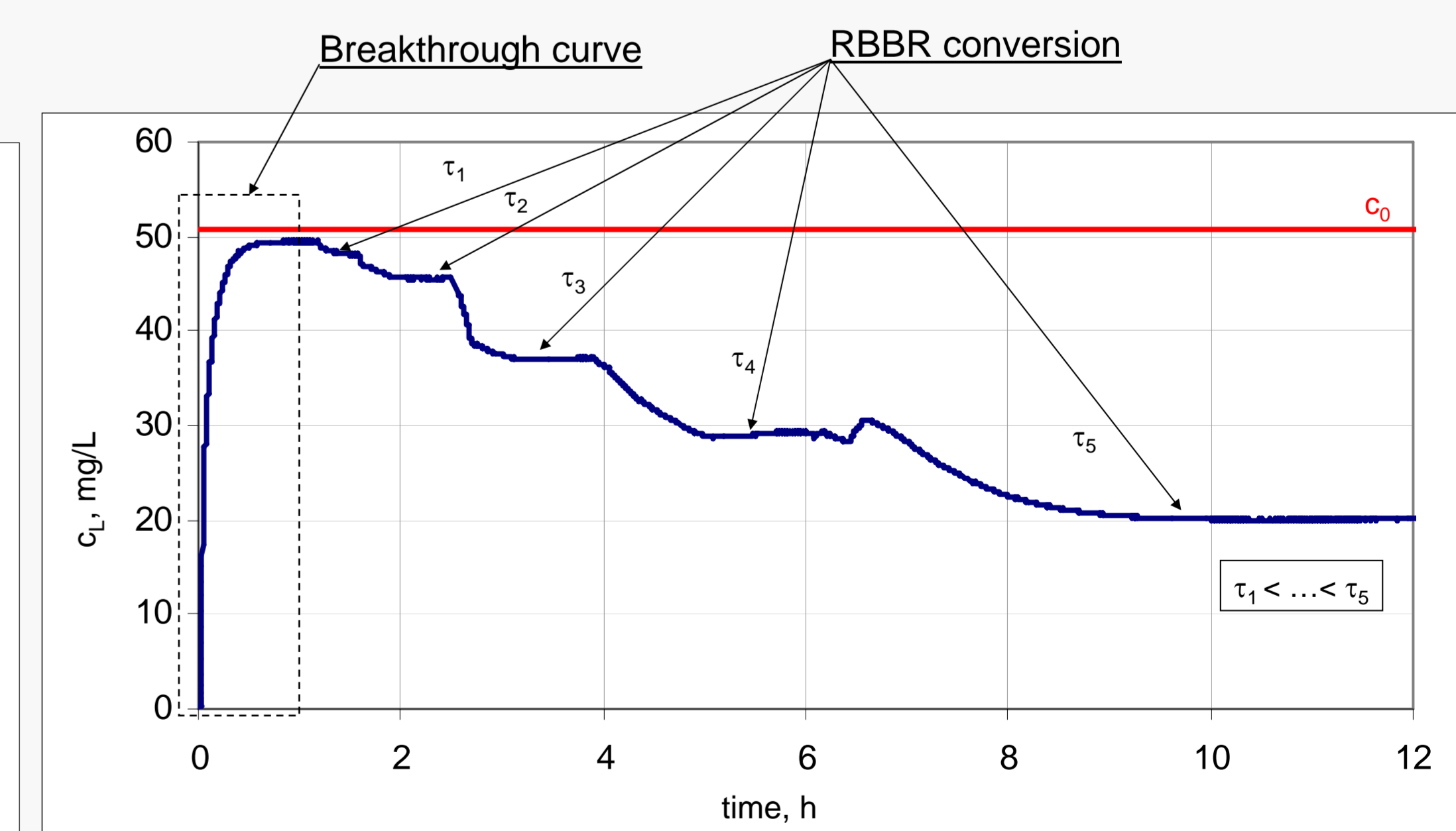


Fig. 3. RBBR concentration at the outlet of the continuously operated reactor (Fig 1B). Total immobilized laccases activity 32 IU.

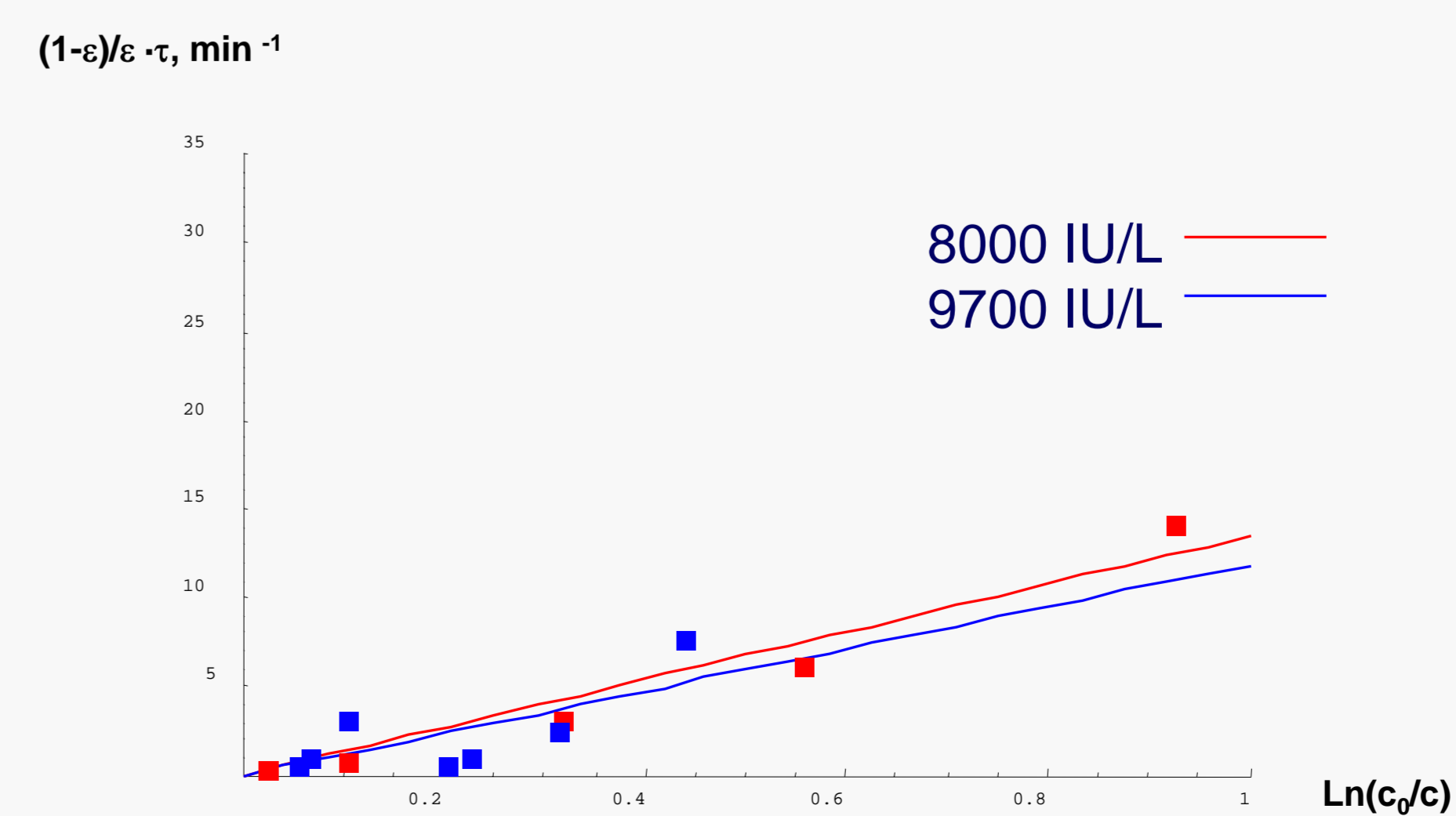


Fig. 4. Assessment of RBBR degradation kinetic by linearized analysis of Eq. 3.

## Bibliography:

- [1] Katchalski-Katzir E. et al., Journal of Molecular Catalysis B: Enzymatic 2000; 10: 157-176
- [2] Palmieri G. et al., Enzyme and Microbial Technology 2005; 36: 17-24
- [3] Hines and Maddox, "Mass Transfer: Fundamentals and Applications" Prentice Hall 1985

**Acknowledgments:** Financial support from EC FP6 Project SOPHIED (NMP2-CT-2004-505899) is acknowledged; the authors thank Dr. Giuseppe Olivieri for helpful suggestions.

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