

**BIODEGRADATION OF 2-CHLOROPHENOL USING FREE AND  
IMMOBILIZED LACCASE FROM *TRAMETES VERSICOLOR***

**SWAPNA SAMBRANI**

**FACULTY OF SCIENCE**

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**APRIL 2009**

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IMMOBILIZED LACCASE FROM *TRAMETES VERSICOLOR***

**SWAPNA SAMBRANI**

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**FACULTY OF SCIENCE  
UNIVERSITI MALAYA  
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**ORIGINAL LITERARY WORK DECLARATION**

Name of Candidate: Swapna Sambrani

(Passport No.: B2583206)

Registration / Matric No.: SGF 060002

Name of Degree: Master of Biotechnology (coursework + dissertation)

Title of Dissertation (“this Work”): Biodegradation of 2-chlorophenol using free and  
immobilized laccase from *Trametes versicolor*

Field of Study: Biotechnology

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## ABSTRACT

Laccase from white rot fungus *Trametes versicolor* was immobilized in sodium alginate beads using entrapment method. Kinetic parameters ( $V_{\max}$  and  $K_m$ ) of both free and immobilized laccase were determined using syringaldazine as substrate. 2-chlorophenol was degraded using free and immobilized laccase. An indirect rapid assay method was developed for the assay of degraded products of 2-chlorophenol, wherein the anionic phenoxy radicals, possibly generated during 2-chlorophenol degradation, couples with a cationic dye, Methylene Blue and renders it colorless. The extent of decolorization indicated the extent of product formation and hence the extent of 2-chlorophenol degradation assuming a 1:1 stoichiometric reaction between degraded products of 2-chlorophenol and the dye. Factors like pH, temperature and enzyme concentration were optimized for the free enzyme reaction and reaction time, temperature and enzyme concentration were optimized for the immobilized enzyme reaction using a statistical tool viz. Box-Behnken method of Response Surface Methodology. The efficiency of immobilized laccase with respect to 2-chlorophenol degradation was found to be comparable to that of free laccase. External mass transfer was found to have negligible effect on the diffusion of substrate from bulk liquid to the surface of the bead while there was significant limitation posed by internal mass transfer from the surface of the bead to the center of the bead.

## ABSTRAK

Laccase dari fungi rot putih *Trametes versicolor* telah dilumpuhkan di dalam ikatan sodium alginate menggunakan kaedah pemerangkapan. Kedua-dua parameter Kinetik ( $V_{max}$  and  $K_m$ ) yang bebas dan laccase yang tidak bergerak telah dikenalpasti menggunakan syringaldazine sebagai substrat. 2-chlorophenol telah didegradasi menggunakan laccase yang bebas dan tidak bergerak. Kepantasan ujian terhadap mutu secara tidak langsung telah terhasil untuk ujian mutu degradasi bahan 2-chlorophenol, sedangkan anionic phenoxy yang radikal, kemungkinan terbentuk akibat dari degradasi 2-chlorophenol, bersama-sama dengan pewarna cationic, Methylene Blue dan peluntur warna. Sejauh mana penghilangan warna menunjukkan sejauh mana formasi bahan terbentuk dan yang demikian degradasi 2-chlorophenol mengangap tindakbalas stoichiometric 1:1 antara degradasi bahan 2-chlorophenol dan bahan pewarna. Faktor seperti pH, suhu dan pemekatan enzim adalah dioptimalkan untuk reaksi enzim bebas dan reaksi masa, suhu dan pemekatan enzim adalah dioptimalkan untuk reaksi enzim yang telah dilumpuhkan menggunakan statistical tool *viz.* kaedah Box-Behnken untuk Kaedah Tindakbalas Permukaan (Response Surface Methodology). Keberkesanan laccase yang tidak bergerak dengan degradasi 2-chlorophenol didapati bersesuaian dengan laccase yang bebas. Pemandahan mass luaran dikenalpasti memberi kesan terabai tentang pembelahan substrat dari cecair yang banyak kepada permukaan rantaian dan didapati juga ada signifikan terhad terhasil daripada pemandahan mass dalaman dari permukaan rantaian kepada bahagian tengah

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