Cross-linked enzyme aggregate systems for degradation of textile dyes

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To preserve the activity of enzymes for biotechnological applications, enzymes are mostly immobilized in/on insoluble supports. The enzymes can be immobilized by different methods such as covalent linkage, adsorption, entrapment and cross-linking of enzyme with soluable polymer using bifunctional agents. The later, cross-linked enzyme aggregates (CLEA's) is a simple technique to produce a biocatalyst with high enzyme activity per unit volume. Since it does not use a solid support for fixation of enzyme, it increases the specific activity of the biocatalyst formed (1-2).

Laccases (polyphenoloxidase, EC 1.10.3.2) mainly produced by plants, fungi, bacteria and insects, are able to catalyze the oxidation of many organic compounds such as methoxyphenols, phenols, o- and p-diphenols, aminophenols, polyphenols, polyamines, molecules from lignin and some inorganic ions (3-5).

In the first part of the study, aggregate formation of enzyme was realized by the treatment with a bi-functional cross-linker in the presence of an appropriate co-precipitator (i.e. soluble polymer with functional group). The enzyme "laccase" aggregate was prepared using chitosan/polyvinylalchol polymer mixture. The known amount of chitosan/polyvinylalchol and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) as a bifunctional cross-linking agent were added to phosphate buffer (100 mM, pH 5), and incubated at 40^{o} C for about 1.0 h with continuous shaking. The prepared CLEAs were characterized to determine the optimum parameters (pH, temperature, reaction time, and shaking speed) of formation aggregates by this new approach. The enzyme aggregates exhibit the most positive features with laccase was selected for further studies. The enzyme aggregate and its free counterpart were compared and discussed with respect to the thermal stability, operational stability, storage stability etc. The CLEAs stability against chemical denaturants was also tested but no significant improvement was detected.

The optimized enzyme aggregates with the highest enzyme activity was applied to degradation of a model textile dye in aqueous solution. To evaluate its potential uses in wastewater treatment as a preliminary step to the removal of organic pollutants in industrial wastewaters for subsequent experiments. In the second part of the study, the performance of enzyme aggregates for the degradation of model textile dye (i.e., Cibacron Blue F3GA) in batch reactors was evaluated. Two phenomena were observed: decolourization of the solutions due to dye adsorption on the support and due to the enzyme action. A high decolourization percentage of practically reactive dye at the beginning and then an effective decolourization of the dye from aqueous solution was obtained, showing the suitability of the immobilized laccase for continuous color removal from textile effluents.

1. Bayramoglu, G. Yilmaz M., Arica M.Y. Reversible immobilization of laccase to poly(4-vinylpyridine) grafted and Cu(II) chelated magnetic beads: Biodegradation of reactive dyes // Biores. Technol.-2010- 101, P. 6615-6621.
2. Sheldon R.A. Cross-linked enzyme aggregates (CLEA's): stable and recyclable biocatalysts // *Biochemical Society Transactions*, 35, -2007- 6, P. 1583–1587.
3. Cabana H., Alexandre C., Agathos S.N.,Jones J.P. Immobilization of laccase from the white rot fungus *Coriolopsis polyzona* and use of the immobilized biocatalyst for the continuous elimination of endocrine disrupting chemicals. // *Bioresour Technol.- 2009*- 100, 14, P. 3447–3458.
4. Bayramoglu G., Arica M.Y. Enzymatic removal of phenol and p-chlorophenol in enzyme reactor: horseradish peroxidase immobilized on magnetic beads. //J. Hazard Mater., -2009-156, 148-155.
5. Celikbicak O., Bayramoglu G., Yilmaz M., Bicak N., Salih B., Arica M.Y. Immobilization of laccase on hairy polymer grafted zeolite particles: Degradation of a model dye and product analysis with MALDI-ToF-MS. // Micropor Mesopour Mater. -2014- 199, P. 57–65.