Immobilization and characterization of a crude preparation of *Escherichia coli* trehalase: A laboratory exercise that integrates concepts of enzymology and molecular biology

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This paper presents a laboratory exercise for teaching enzyme technology through an approach that integrates concepts of enzymology and molecular biology. This Laboratory Exercise is based on the extraction of trehalase (EC 3.21.28) from a *Escherichia coli* strain Mph2 (carrying the plasmid pTRE11 that harbors the gene *TreA*+) and immobilization of the enzyme on chitin. Therefore, the students participate in bacterium cultivation, protein extraction from *E.coli* periplasmic space, preparation of chitin particles and enzyme immobilization. Trehalase was chosen because it can be easily released by osmotic shock and it is not necessary to purify it from the osmotic shock fluid (OSF) because it is free of any other glycosidase. This lab exercise also teaches how to determine the kinetic parameters (Michaelis constant- Km and maximum velocity- Vm) for free and immobilized enzymes. It is obvious that the determination of kinetic parameters using the Michaelis-Menten model is not a novelty. Nevertheless, an experimental class that includes the comparison of these parameters for free and immobilized enzymes, using the students´ own experimental data, would be extremely useful for teaching the subject. The students can identify and compare these parameters, as well as discuss the differences and similarities among them. This discussion points towards issues such as enzyme conformation and structural rigidity related to changes in kinetic parameters mainly Km. This Laboratory Exercise was designed for graduate students that have a good background in Biochemistry and have joined courses involving either Enzymology or Enzyme Technology.