Functional Clay Nanotube-Enzyme Biocomposites

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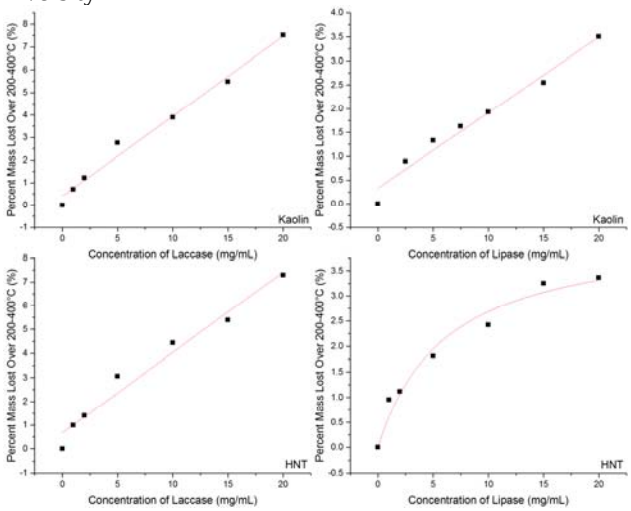
Statement of Purpose: Proteins are a diverse group of biopolymers that serve as the backbone of functionality in nature. However, having diversity does not exclude proteins from serving a specific function. Enzymes are a particular class of proteins that serve as catalysts for chemical reactions. Enzymes exhibit extreme efficiency and specificity at the cost of sensitivity to their environment. Most enzymes cease to function in non-aqueous environments and are sensitive to both pH and temperature. Furthermore, enzymes are soluble and therefore difficult to reuse. To overcome some of these particular disadvantages, researchers have immobilized enzymes on solid substrates. Immobilization facilitates reuse and may imbue additional pH or temperature stability to the enzyme at the cost catalytic efficiency. Enzyme immobilization on clay substrates has been studied at least since 1938[1]. In this study, the enzymes laccase from *Trametes veriscolor* and lipase from *Candida rugosa* were been immobilized on halloysite nanotubes (HNT). Halloysite nanotubes are a morphological derivative of the clay kaolinite. The nanotubes have an average diameter of 50 nm, average length of 300 nm, and lumen diameter of 15 nm. Quantification and analysis of adsorption, desorption, and enzyme catalysis at various pH was performed in order to assess halloysite nanotubes as an enzyme immobilization substrate.

Methods: Halloysite nanotubes were provided by Applied Minerals Incorporated from their Dragon Mine in Utah. Lipase (EC 3.1.1.3) from *Candida rugosa*, laccase (EC 1.10.3.2) from *Trametes versicolor*, kaolin, fluorescein diacetate (FDA), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and sodium azide were bought from Sigma Aldrich Corporation, St. Louis Missouri. The proteins were immobilized onto halloysite and kaolin by incubation in solution and vacuum treatment. Enzyme desorption was studied using UV-vis spectroscopy. The absorbance at 280 nm was measured as a function of time at discrete points. Enzyme adsorption was studied using thermo gravimetric analysis. The enzymes showed decomposition generally within the temperature range of 200-400 C. This range was used as the standard for assessing the mass percent lost in composite samples. Laccase activity was assessed using ABTS as an oxidation substrate. ABTS is a colorless substrate that absorbs light at 420 nm after oxidation by laccase[2]. Lipase activity was assessed by FDA hydrolysis[3]. FDA becomes fluorescent after hydrolysis of the acetate groups by lipase. The rate of fluorescent intensity was used as the parameter for comparison. The enzyme assays were performed at various pH in order to assess the pH stability of the immobilized and free enzyme.

Results: Proteins can be loaded and released from halloysite nanotubes. Proteins can be immobilized at 4-8 wt% even after washing. When placed into water, 25% of



the immobilized protein will desorb over a period of 24 hours with an initial burst of 80% of the 25% occurring in the first hour. The desorption kinetics can be described by first order kinetics or the power law with burst release taken into account[4]. Adsorption kinetics are protein and substrate dependent. Lipase when adsorbed onto HNTs can be described with a Langmuir adsorption isotherm. When adsorbed onto kaolin, lipase shows linear adsorption kinetics. Laccase shows linear adsorption on both HNT and kaolin. Both enzymes show enhanced pH stability at acidic pH when immobilized, but lower catalytic activity overall. Immobilized lipase retains greater catalytic activity at pH less than 7.4, but only for the first day when compared to the free enzyme. Laccase shows greater temporal stability at acidic pH and no difference at basic pH when compared to the free enzyme.

Conclusions: Halloysite nanotubes can be used as an immobilization substrate which does not require any special procedure for immobilization, provides both long term and short term availability of enzyme, and enhances the acidic pH stability of the enzyme at the cost of catalytic activity. Halloysite composites can be reused by filtering or centrifuging the sample as halloysite readily precipitates. The improved stability may be due to a variety of factors including reduction of possible conformation due to immobilization, localization inside of the nanotube, and

References:

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