DYNAMIC NANOCOMPOSITE SELF-DEACTIVATING FABRICS FOR THE INDIVIDUAL AND COLLECTIVE PROTECTION

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Chemical and Biological Defense (CBD) is of high Army and National military priority. Battlefield protective uniforms and shelters must simultaneously meet weight, bulk, durability, comfort and heat stress requirements for a combat configuration. The JSLIST Suit, first introduced in 1997, is a remarkable garment used by all branches of the U.S. military that meets many of these requirements. The current JSLIST suit is comprised of an outer shell that is a nylon blend which imparts tear strength and

providing a barrier protection or adsorption of warfare agents, or a combination of both. These mechanisms suffer from an inherent tradeoff between breathability/comfort and protection levels, and place significant burden on doffing procedures in an actual event.

Foster-Miller and BHA Holdings Group have developed a nanocomposite fabric architecture to enhance the current capabilities by incorporating a "self-deactivating"
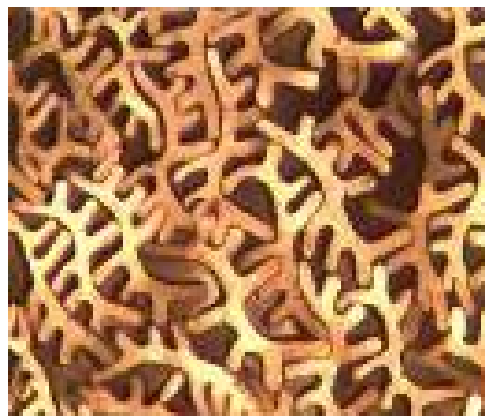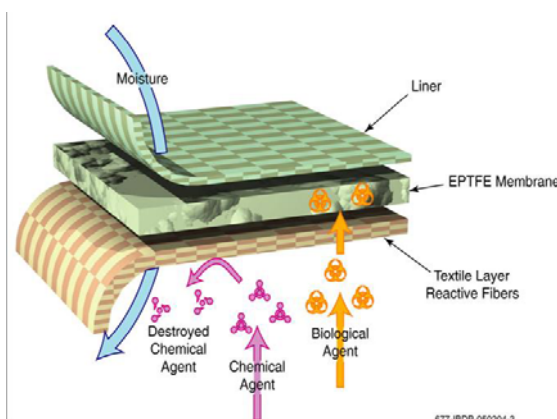

Figure 1: Nanocomposite multiplayer architecture (left) for self deactivation of chemical and biological threats. Cross section of the high surface area capillary channeled (C-CP) fibers (right)

durability to the suit. The hung liner consists of beads of activated carbon laid down in adhesive form onto a non-woven front textile to provide a method of adsorbing threats such as nerve and mustard gases, and prevents the soldier from experiencing percutaneous exposure to these toxins. Despite the usefulness of the current JSLIST suit, there are some improvements in the suit that could make the suit even more effective. These include protection against a broader range of chemical agent threats; bioaerosols and toxic industrial chemicals/toxic industrial materials (TICs / TIMs). Current and emerging chemical defense garments and textiles are based on

functionality and bioaerosol protection into a fabric. As shown in Figure 1, the new design has pioneered the use of threat destroying catalysts directly incorporated into the nanoreticulated textile and the micro porous membrane components of the laminate system. Thus, a self-decontaminating material will eliminate the risk of exposure during doffing procedures and improve the overall effectiveness of the system. In addition, a micro porous membrane capable of blocking biological threat agents has been laminated into the Foster-Miller/BHA material. These important functionalities are added into the nanocomposite and significantly improve the

	Form Approved OMB No. 0704-0188
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1. REPORT DATE **01 NOV 2006**	2. REPORT TYPE **N/A**	3. DATES COVERED **-**	
4. TITLE AND SUBTITLE **Dynamic Nanocomposite Self-Deactivating Fabrics For The Individual And Collective Protection**		5a. CONTRACT NUMBER	
		5b. GRANT NUMBER	
		5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)		5d. PROJECT NUMBER	
		5e. TASK NUMBER	
		5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Foster-Miller, Inc. 195 Bear Hill Road Waltham MA 02451**		8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		10. SPONSOR/MONITOR'S ACRONYM(S)	
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT **Approved for public release, distribution unlimited**			
13. SUPPLEMENTARY NOTES **See also ADM002075., The original document contains color images.**			
14. ABSTRACT			
15. SUBJECT TERMS			

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT **UU**	18. NUMBER OF PAGES **8**	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**			

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

comfort / wearability of the garment and its overall mechanical properties.

The initial challenge was described as the defense of known and unknown threat agents. To address this we have developed a simulation tool to accept generic input parameters that will describe the efficacy of individual layers and the composite system against both known and unknown chemical and biological gaseous, aerosol, and liquid threats. The nanocomposite material is being modeled such that the simulation of performance matches known experimental results and drives selection of the component materials. The tri-layer system consists of a textile outer layer, a membrane middle layer, and an inner liner layer. The outer layer is a textile that is created from very high surface area "reactive" Capillary Channeled Polymer (C-CP) fibers from Specialty Custom Fibers, LLC of Clemson, SC. These fibers have been embedded with catalytic materials (shown in Figure 1). The reactive fiber layer has been effective destroying chemical threat agents. The second layer is an expanded poly(tetrafluoroethylene) (e-PTFE) membrane. The pore size in this membrane has been manipulated such that aerosols can be entrapped in the membrane preventing biological agents from reaching the intended target. The membrane is treated with catalytic polymers to add a secondary level of protection against chemical threat agents. Finally, an inner lining layer, worn closest to the body, contains an activated carbon felt to serve as a breech layer in the event it passes through the first two layers.

In this paper we will discuss recent developments we have made to our catalytic polymer substrate, which serves as a central, flexible building block for our multilayer architecture. The catalytic polymer substrate can be applied to a variety of fabrics, including ePTFE membrane, nylon Capillary Channeled Polymer (CCP) fibers, and may be applied to both in the final application. It can also carry more than one enzyme catalyst so that it can protect multiple types of threat agents. Finally, it has high sorption capacity which will reduce the hazards associated with agent breakdown products.

1. BIOLOGICAL CATALYSTS

The catalytic system we have introduced is a combination of three enzymes. This catalytic system is comprehensive, providing protection against nerve and blister agents as well as biological agents. The system will endure multiple challenge cycles. In addition, through the use of poly-ß-cyclodextrins (PCDs) and poly-trehalose (PTH) as polymeric supports, the incorporated enzymes will be able to repair themselves through a re-folding process. The catalytic system is deposited on fibers and membranes using layer-by-layer assembly technique. [ref]

1.1. Uniqueness of our specialty enzymes

An ideal catalytic system for chemical decontamination should provide full protection against nerve and sulfur mustard agents. For that purpose, organophosphorous hydrolase (OPH), organophosphorous acid anhydrolase (OPAA), and haloalkane dehalogenase (HD) are being used as polymeric particles in fabrics. OPH and OPAA are organophosphate-hydrolyzing enzymes are the most effective biocatalysts to degrade chemical nerve agents rapidly (G and V series respectively at ~50 sec^{-1}). Genencore, Inc is now successfully producing this enzymatic decontamination technology under the trademark DEFENZ[TM]. The sulfur mustard degrading haloalkane dehalogenase (HD) is the first enzyme developed to degrade sulfur mustard within a few seconds [6.9 sec^{-1}]. In addition to its rapid decontamination, it has been shown that the decomposed by-products of this enzyme on sulfur mustard are not toxic and are not genotoxic, unlike intermediates formed through conventional slow abiotic hydrolysis [0.0046 s^{-1}] which contain sulfoxide functionality.

1-2. Enzyme immobilization *via* layer-by-layer assembly

Enzymes are generally unstable and deactivate rapidly. They have limited shelf life in solution, and their performance as a catalyst is limited to a small temperature and pH range. Many strategies for immobilization have been pursued in recent years to stabilize enzymes, and to retard their deactivation upon exposure to stressful conditions such as rain, heat, and daylight. The layer-by-layer assembly via electrostatic, non-covalent adsorption has been an attractive process for this work, owing to its

non-intrusive nature, simplicity, and the fact that it is an inexpensive method of fabrication.

Layer-by-layer assembly of enzymes have been reported to retain their catalytic activity over long periods of time (> 6 months). Organophosphorous hydrolase (OPH) (50 sec^{-1}, 15 µg VX/ml) was immobilized between layers of buffered polyelectrolyte on silica microspheres. [ref] When immobilized between molecular layers, the OPH maintained a higher catalytic activity. Without immobilization, it denatures within 62 h, and all enzyme activity is lost. Cotton cloth with OPH enzyme fabricated by similar techniques retained its hydrolytic activity against methyl parathion (MPT), and a strong yellow color developed as a result of rapid catalytic degradation within five minutes of exposure. [ref] V agent degrading OPAA enzyme was also demonstrated to have exceptional hydrolytic activity against a VX simulant when immobilized onto sorptive polyurethane. [ref] HD enzyme immobilized cotton thread also facilitated the release of HCl (hydrochloric acid) immediately upon exposure to chloroethyl methyl sulfide (CEMS) in aqueous medium at ambient condition. The isoelectric point of the three enzymes are all effective at pH 6.4-7.5, where all three enzymes could be incorporated sequentially into polymeric substrates, and serve as a comprehensive countermeasure to have all G, V, and H agents decontaminated upon encounter.

1-3. Enzymatic chaperones facilitate enzyme self-repair

Though tuned favorably to local microenvironment, our enzymes immobilized within the polymer matrix degrade gradually after cycled use over time under stressful conditions. Thus a need exists to regain high turnover rates to their initial states for continuous degradation. We have used poly-ß-cyclodextrin derivatives bonded with BPEI/Erkol polyelectrolyte, as a molecularly layered unit to protect the enzyme. This construction should serve as a chaperone within the molecularly-confined local area to revive enzymes with lowered activity levels that served beyond their cycle use time.

Artificial enzyme chaperones have emerged to revive lowered activity of denatured enzymes. Enzymes are amphoteric by nature, having specific molecular domains and hydrophobic surfaces that trigger more complicated intermolecular or inter-domain interactions, ultimately leading to the denaturing of the enzymes. They tend to aggregate upon exposure to stressful conditions such as heat, light, salt, and extreme pH. Denatured lysozyme (hen egg white lysozyme, HEWL) was found to regain 75 % of its original enzyme activity by using ethylammonium nitrate as a chaperone of which 90 % of the active lysozyme was recovered at dilution protocol. Re-folding of lysozyme has been reported by assistance of cyclodextrins bonded to titania nanoparticles. Harding and his research group have reported that inactivated glucose-6-phosphate dehydrogenase was re-natured, using 6-aminohexanoic acid as a chaperone, stabilizing the native conformation of proteins.
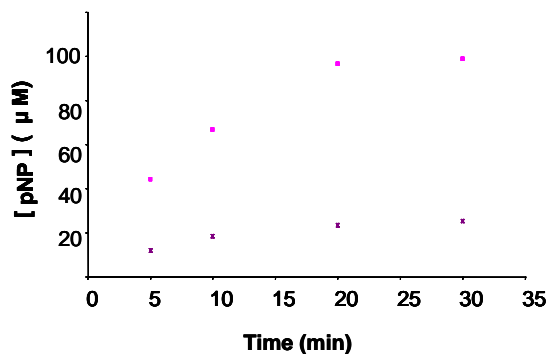

Fig. 2 Kinetics of used OPH cotton thread before (purple) and after (pink) chaperone treatment: Increase of pNP concentration is indicative of "used" enzyme thread

Refolding of immobilized enzymes is crucial to the practical use of enzyme fabrics. Poly-ß-cyclodextrin implemented cotton thread (1 m long) was coated with OPH enzyme (pH 8.6) by known procedure and used to degrade freshly prepared MPT solution, which was recorded as the lower curved kinetics (20 µM pNP after 30 min) in Fig 2. After its use, the OPH thread was rinsed using CHES buffer briefly to eliminate MPT drops left on the thread. A few drops of CHES buffer were added to the bottom of the scintillation vial container, and then placed in a freezer (7 °C) for 2 days. After 48 h, the OPH cotton thread was withdrawn from the freezer and exposed to a fresh 10 mL MPT solution. We monitored its subsequent catalytic

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MPT degradation of the used OPH thread. Interestingly, the amount of pNP produced in the bulk increased enormously, almost by a factor of 5 (~ 100 µM after 30 min), indicating that catalytic sites of OPH cotton thread were re-activated using CHES buffer and poly-ß-cyclodextrin as a chaperone

This experimental result of enhanced activity using the poly-ß-CD and layer-by-layer assembly was also reproduced on OPH coated nylon substrates (Biodyne B-membrane procured from Pall corporation), when coated with OPH enzyme. It might be premature, but it could be concluded that once enzymes are immobilized in a molecular assembly of polyelectrolyte, an artificial chaperone could serve to re-nature enzymes within the catalytic multilayer system, similarly as the effects of AthenaES[TM]'s QuickFold[TM] commercially available product.

2. PHYSICAL SUPPORT : POLY-ß-CD

2.1. Synthesis of poly-ß-cyclodextrin derivatives (Poly-ß-CD)

Environmentally benign, light weight polymeric β-cyclodextrins (PCD) and its derivatives are known to sequester volatile organic chemicals (VOC) and also to capture into their cavities biological species through inclusion complex formation within their interior hydrophobic core. Incorporation of β-cyclodextrin into porous polymers might impart
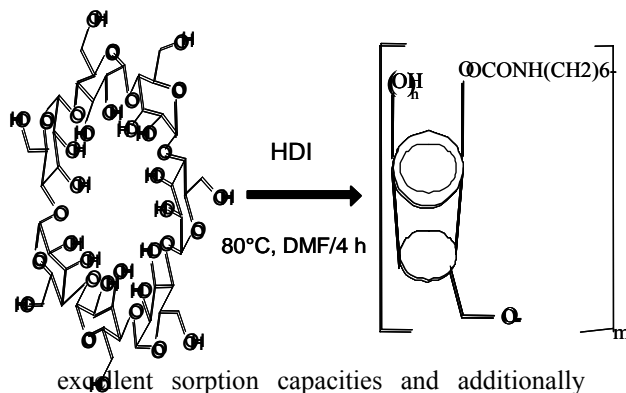

Fig. 3 Polymeric ß-cyclodextrin particles

could achieve clean air chemistry by decontamination followed by sequestration when biological catalysts are incorporated into the polymers. In our previous study, one of the poly-ß-cyclodextrins (Poly-ß-CD) was prepared *via*

exhaustive cross-linking of ß-CD with hexyl diisocyanate (HDI) in DMF at 80 ºC for 4 h, sonicated in MeOH (Fig 3). It was molecularly sieved (32-53µ) and its surface morphology was characterized by SEM (Fig. 4).

The particles were characterized by FT-IR [CO_2 group at 1715 cm^{-1}], BET surface area: 10.2 m^2/g, The total pore volume is 0.06 cm^3/g. Such pollutants as methyl parathion (MPT) and *p*-nitrophenol (pNP) were sequestered by forming inclusion complexes with pNP with formation constant (K)=10^9 . The formation constant for ß-CD is K=10^2. Conveniently, enzymatic status was recovered and regenerated by simple immersion in methanol. Its preferential sorption to MPT over pNP was also observed in a batch mode. These polymeric particles were shown to be chemically inert and robust enough to endure extended exposure to highly acidic and highly alkaline media.
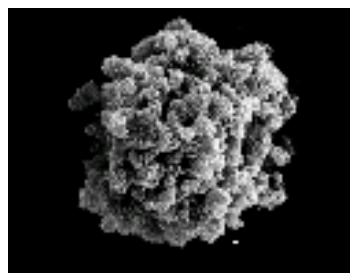

Fig. 4 SEM image of sieved Poly-ß-CD particles with large surface area [~10 µ] [ref]

2.2 Sorption reinforced catalytic degradation of pesticides and chemical agents

The exhaustive cross-linking of ß-cyclodextrin derivatives with excessive amounts of alkyl spacers (more than 8 eq of HDI) was not a desired method to produce the highest absorbing for both MPT and p-nitrophenol (pNP) , its degradation product. This method does not produce the best physical support for an enzyme catalytic system. Even self-cross linked hexyl diisocyanate (HDI) particles were demonstrated to have decent sorption capacity toward *p*-nitrophenol (pNP) through hydrophobic interaction. The preparation of polymeric ß-cyclodextrin derivatives should be carefully controlled by molecular ratios (mass) so as to generate *in-situ* the adequate amount of ß-

cyclodextrin imbedded in the cross-linked polymeric matrix.

When reacted with large excess of Desmodour®, trimeric HDI, poly-CD particles were not demonstrated to have pNP adsorbed on particles, indicating that all molecular buckets of ß-CD unit were completely blocked with bulky alkyl spacers to reject host-guest interaction with pNP from aqueous medium.

catalytic capability (twice the previous condition of 8 equivalents of HDI employed).

Based on the experimental results, the optimal equivalent molar ratio of β-CD to HDI was determined to be 1:4, and this optimized reaction system was reproduced onto cotton threads, erkol-treated ePTFE membranes, and fibrous cellulose.
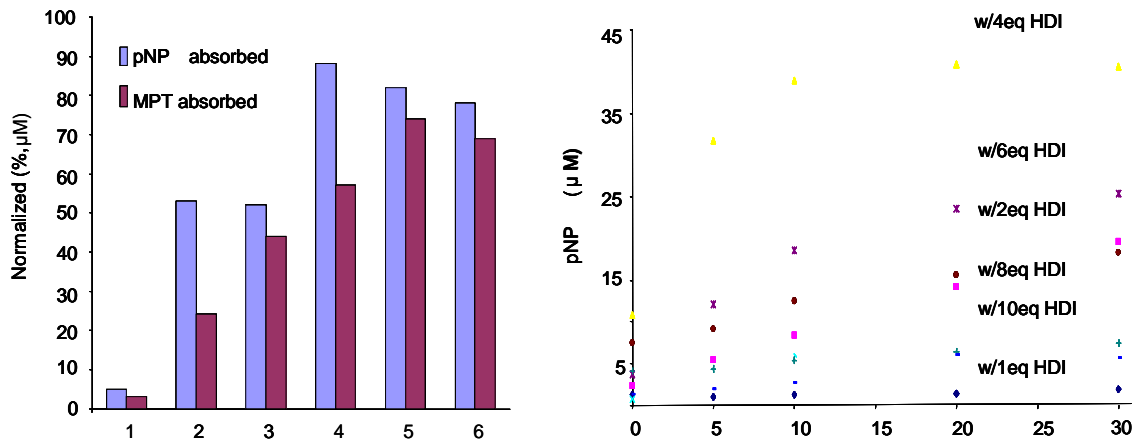

Fig. 5 Poly-ß-CD-cotton fiber for sorption (left) and OPH coated poly-ß-CD cotton fibers for catalytic MPT degradation (right)

In addition, 20 cotton threads (50 cm long) were modified individually with ß-CD *in-situ* by altering their equivalent mass of hexyl diisocyanates [i.e., equivalences of ß-cyclodextrin vs hexyl diisocyanate: 1 eq. vs 1 eq, 1eq. vs 2 eq., 1 eq. vs 4 eq., 1 eq. vs 6 eq., and 1eq. vs 8 eq.] Each sample's sorption capacity against pNP and MPT was tested. Similar to before, each ß-CD implemented cotton string was coated with OPH under the same condition, and its enzymatic activity was assayed using UV-VIS absorption spectra of pNP [405 nm]. The amount of enzyme loaded could also be maximized in this controlled quantitative deposition method.

Maximized catalytic degradation is desired and could be achieved from maximum loading of enzyme, by monitoring maximum absorption of MPT and its degraded product, pNP. *In-situ* modification of cotton threads (1 m long) were carried out and coupling of ß-CD with 4 equivalents of HDI had the best sorption and
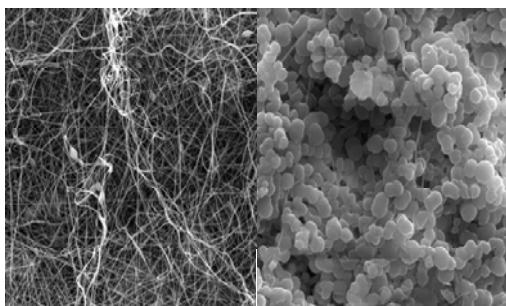
2.3 Performance of Poly-ß-CD as sorption materials of pNP and MPT

Cross-linked poly- ß-cyclodextrin (PCD) has been shown to effectively absorb pNP from aqueous medium. [ref] In a batch mode, the absorbing properties of PCD were evaluated over a range of pNP concentrations (0.05-50 mM) at a buffered solution pH of 8.6 and 10.0, demonstrating maximum experimental capacities of 22 and 195 mg/g PCD, respectively. Under the conditions studied, pNP uptake could be accurately described using the Sipps equation ($R^2 \geq 0.998$), providing an average apparent binding affinity for pNP of 7.15 and 53.76 mM at pH 8.6 and 10.0, respectively. In a continuous flow-through system, PCD performs similarly as batch mode. Sorption capacity increases with increasing influent concentration of pNP and with decreasing pH. At slower flow rates, Poly-ß-CD exhibits a sharper break-through, and column regeneration can be achieved with a few bed volumes of ethanol.

Sorption-capable Poly-ß-CD have been prepared not only for absorbing chemical agents through inclusion phenomena, but also for acting as insoluble supports to hold enzymes that degrade the pesticides. As a supporting material of the OPH enzyme in our enzyme catalytic system, poly-ß-cyclodextrins (Poly-ß-CD) are being suggested due to their high surface area for sorption capability [K (M^{-1}); 5 x 10^9 for poly-ß-CD versus 3.4 x 10^5 for charcoal][ref].

2.4 From microparticles to nanoparticles of poly-ß-CD

Coupling reactions of cross-linkable monomeric units are hardly controlled because it's highly exothermic once it hits its activation energy and can't be stopped as desired. Utilizing optimized reaction conditions at dilute concentration in DMF, nanoparticles of poly-b-CD were produced and imbibed onto the nanofibrous polymeric mats over 14 h. With this protocol, we could have nano-sized cross-linked polymeric β-CD particles fabricated using

electrospun nanofibers as a template.

Super-absorbing poly-ß-CD was intercalated between polyvinyl alcohol (PVA) electrospun nanofiber when treated in-situ with a mixture of ß-cyclodextrin hydrate and hexamethylenediisocyanate at 70 °C. PVA nanofiber samples were electrospun on aluminum foil. It was treated with polymeric cyclodextrins [similar to above procedure], and rinsed with hot DMF. A large amount of poly-ß-

Fig. 6 SEM of PVA electrospun fibers treated with polymeric ß-cyclodextrins

CD nanopororous particles were imbibed into the PVA nano-fibers.

Cross-linked PCD nanoparticles on PVA nanofibers are insoluble, template initiated polymerized, and super-absorbing with large

surface area. They are self-decontaminating and recyclable upon enthalpy change (from aqueous to organic solvent change for safe disposal), environmentally benign, and highly catalytic (when OPH was incorporated) against MPT.

3. PHYSICAL SUPPORT ; POLY-TREHALOSE (PTH)

In a similar manner, polymeric trehalose (PTH) was also prepared in a different molar ratio optimized to maximize catalytic sequestration of pesticides and chemical agents. Unlike the poly-ß-CD particles, due to intrinsic hydrolytic capability toward methyl parathion (MPT), polymeric trehalose (PTH) particles were prepared as a complementary substrate to poly-ß-CD particles in various aspects serving as an additional physical support for enzyme catalysts.

These environmentally-benign, polymeric particles were demonstrated to have high binding efficiency for the enzyme catalyst, high enzymatic activity, the capability to efficiently hydrolyze and sequester their substrates, and the ability to act as sorption-induced hydrolyzing vehicles against biological and chemical threat agents.
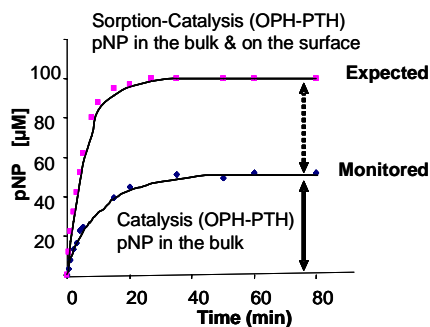

Fig. 7 Catalytic Degradation of MPT employing OPH-PTH cotton thread

D-(+)-Trehalose, a disaccharide widely employed for enzyme stabilization, was incorporated into the polymer matrix generated *in situ* on cotton thread by coupling reactions with alkyl (aryl) isocyanate derivatives. These functionalized cotton threads were chemically inert and robust enough to endure extended exposure to stressful conditions. They were demonstrated to absorb organic pollutants such as methyl parathion (MPT) and *p*-nitrophenol (pNP) in aqueous medium. Upon exposure to

methanol, these compounds were released immediately into the organic medium for safe disposal and thereby the functionalized cotton threads were recovered for reuse.

Fig. 8 OPH-PTH cotton thread recovered before (left) and after (right) MPT degradation

The degradation of MPT was assayed as the amount of pNP produced in the bulk. 42 uM pNP was the maximum pNP produced and 58 uM pNP was believed to be adsorbed to the surface of cotton thread by inclusion phenomena and hydrophobic interactions.

4. Complementary supporting materials of enzyme : Poly-ß-CD (PCD) and Poly-Trehalose (PTH)

When both OPH-PTH and OPH-PCD substrates were compared in terms of catalytic performance, they were demonstrated to have high enzymatic activity sustained under stressful conditions. However, stronger absorbing capability of OPH-PTH were demonstrated than that of PCD, though not regenerated enough for safe removal upon the enthalpy change by replacing water with methanol. Substantial hydrolytic capability of OPH-PTH was also unexpected. Both OPH-PCD and OPH-PTH particles and substrates could be employed for desired application.

Fibrous cellulose was employed as a physical support of organophosphorous hydrolase (OPH). The enzymes were held in molecular layers of buffered polyelectrolytes adsorbed sequentially, constructed through electrostatic interaction without distorting the enzyme architecture. These enzyme friendly trehalose/OPH/polyelectrolytes bearing cellulose fibers are an efficient platform for destroying biological and chemical threat agents.

5. SORPTION REINFORCED CATALYTIC SYSTEMS

Several versions of the catalytic system have been prepared and tested against a simulant of chemical nerve agent. OPH enzyme modified CCP fibers and OPH enzyme modified nylon membranes also show rapid hydrolytic degradation of methyl parathion (MPT). Cotton modified with PCD and OPH enzyme was also prepared and showed improved sorption and sorption reinforced catalytic performance. Cross-linked poly-trehalose (CPT) particles were prepared which have further advantages: D-(+)-trehalose stabilizes enzymes can hydrolyze MPT in its cross-linked polymeric substrates unlike PCDs, and has higher sorption capacity for both MPT and pNP. The advantage of the architecture that we have employed is that as more efficacious catalysts are discovered they can be easily incorporated into the nanocomposite and should the threat size change the porosity of the nanocomposite material can be manipulated to satisfy the thret.
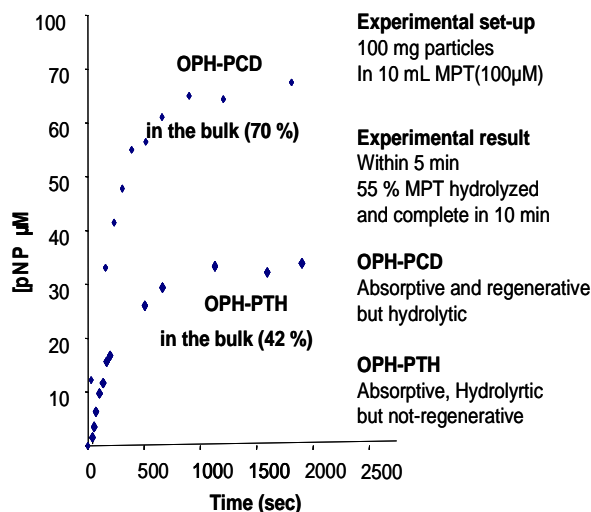

Fig. 9.Catalytic performance of OPH-PTH cotton thread vs OPH-PTH cotton thread

Our enzyme based catalytic system was demonstrated to be promising when coupled with absorbing polymeric cross-linked particles. It was further demonstrated to be highly catalytic under stressful conditions such as salt, ionic strength, acid, alkaline, and organic solvents. Under stressful conditions OPH-PCD particles were demonstrated to sustain enzyme activity, meaning that stressful conditions can be

overcome by the molecular interaction of enzyme with absorbing polymeric particles, and its performance as catalytic system could be improved by chemical modification of supporting polymeric materials.

ACKNOLEDGEMENT

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