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Novel Approach to Controlled Surface Modification in Textile Via Magnetic Cross-Linked Enzyme Aggregates (Clea)

Tekstilde Çapraz Bağlı Enzim Topluluklarının Kontrollü Yüzey Modifikasyonunda Kullanılması

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NOVEL APPROACH TO CONTROLLED SURFACE MODIFICATION IN TEXTILE VIA MAGNETIC CROSS-LINKED ENZYME AGGREGATES (CLEA)

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ABSTRACT: In this proceeding, architecting novel magnetic Cross-Linked-Enzyme-Aggregates (CLEA) nanoparticles to effectively and controllably modify textile surfaces without damaging the fabric itself, will be discussed. The efficiency is due to exponentially increased surface concentration of biocatalyst and to the choice of aggregate size which controls the penetration depth and hence preventing the fabric disruption. In addition, due to the presence of magnetic nanoparticles, the continuous recovery and reusability makes this design more economic and ecologic compared to the conventional chemical processes. The careful fabrication and functionalization of such a design with preliminary results will be presented in this contribution.

Keywords: Biocatalysis, enzymatic process, biomimicking, biostoning, textile surface treatment, cellulosome, clea, magnetic nano-particle, reusability

TEKSTİLDE ÇAPRAZ BAĞLI ENZİM TOPLULUKLARININ KONTROLLÜ YÜZEY MODİFİKASYONUNDA KULLANILMASI

ÖZET: Bu makalede, manyetik nano-parçacıkların da içinde bulunduğu, çapraz bağlı enzim topluluklarının (CLEA) oluşturulması ve tekstil işlemede kullanılması fikri ve uygulaması sunulmuştur. CLEA'nın, serbest enzimlere göre daha verimli olmasının iki temel nedeni vardır. Birincisi ayarlanabilen CLEA büyüklüğü ile enzim etkinliğinin kumaşın gözeneklerine girme miktarı sınırlandırılabilir ki bu, kumaşın mukavemetini artırır. İkincisi ise, enzimlerin kumaşın istenmeyen bölümlerine girişini engelleyerek, yalnızca yüzeye yönlendirilmeleri sayesinde yüzey işlenme veriminin arttırılmasıdır. Bunlara ek olarak, CLEA içine yerleştirilmiş manyetik nano-parçacıklar CLEA'nın tekrar tekrar kullanılmasına imkan sunar. Önerilen süreç, alışılagelmiş kimyasal süreçlerle karşılaştırıldığında daha tutumlu ve çevrecidir. İlk CLEA tasarımımız ve uygulamaları, öncül ölçüm sonuçları ile beraber sunulmuştur.

Anahtar Kelimeler: Biyokataliz, enzimatik proses, biyo-taklit etme, biyotaşlama, tekstil yüzey muamelesi, selülozom, clea, manyetik nano-partikül, tekrar kullanılabilirilik

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1. INTRODUCTION

Enzymes are biological catalysts; accelerate the rate of chemical reactions without themselves undergoing any chemical changes. They are closely utilized in numerous biotechnological processes and widely applied in many different industries and the number of applications continues to increase. The main reason why enzymes are getting popular in industry is due to the fact that they are environmentally friendly as proteins bio-degrade naturally without harming the environment, substrate specific unlike chemicals and can replace many chemical reactions at once.

Enzymatic specificity means that the enzymes are programmed to function only on a set of specific substrates and leave everything else around intact. It is analogous to a key-and-lock mechanism. Enzymes perform reactions of organic chemistry possible in *the most efficient* manner as they can replace multiple chemical steps at once. Additionally, they do not require *extreme conditions* such as very low or high pH and temperature values, making it easier to use and less expensive -in these terms-.

In the past, the finishing of denim garments has been revolutionized by the application of enzymes [1, 2] as an alternative to the pumice stonewashing process. Until now, acid cellulases have been heavily used in stonewashing, stoneless washing processes (biostoning) to impart various effects to the fabrics in terms of contrast, shade and smoothness [3], however, the cellulases [2, 4, 5] that are currently applied in textile processes still require improvement in the following areas:

(i) Mechanical action such as in jets or rotating drum washers

(ii) Controlled deactivation to reduce the tensile strength loss (TSL) induced by cellulase action [6],

(iii) Controlled back staining from the indigo particles released during cellulase washing [7]

Point (iii) has raised a fair amount of research articles since, and besides cellulose binding domain, certain hydrophobic sites and other non-polar surfaces available in the cellulases interact, bind indigo dye molecules and act as an emulsifier, helping the dyes to float out of the cellulose fibres during hydrolysis [8, 9]. It is only through the understanding of the cellulase interactions to both cellulose and indigo that one can *rationally* design a suitable system for efficient biostoning.

The focus of our work is on the fabrication and functionalization of cross-linked cellulase(s)/glucose oxidase aggregates to form particles that can controllably removes indigo dye molecule from denim without the fabric destruction. Cellulases are multi-component enzyme systems and a complex mixture of Endoglucanases (EG), Cellobiohydrolases (CBH), Cellobiases, where all these components work synergistically in the following manner: EG hydrolyse cellulose randomly along the chains, preferentially the amorphous region, CBH attack the chain ends and produce primarily cellobiose coupled with the enzyme. The cellobiose and any small chain oligomers produced by CBH are then hydrolysed by cellobiase into glucose molecules. In addition to cellulases, introducing glucose oxidase within the CLEA architecture to consume the glucose molecules produced by the cellulase enzyme cocktail, will produce hydrogen peroxide which, in turn, will add additional value to the biostoning processes (Figure 1). Therefore, a clear understanding of individual enzymes' functions is crucial for the selection of CLEA design.

2. METHODOLOGY

Mimicking cellulosomes-like function in a synthetic aggregate is considered to be promising. Literature demonstrated that the potential of using immobilized and/or co-immobilized cellulases for the controlled process of bio-stoning is feasable [10-12]. However, the performing enzymes often lack the control of denim mechanics, back staining and recoverability. The following subsections will detail these concerns thoroughly.

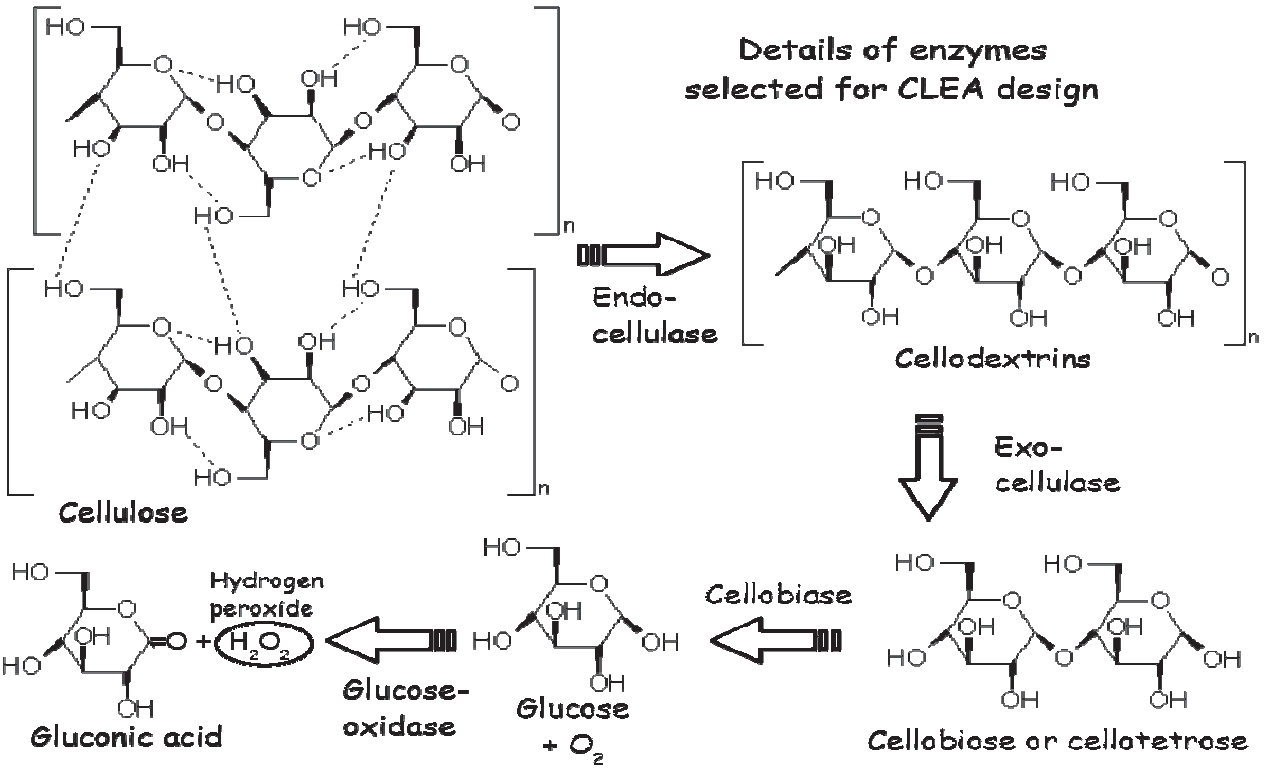

Figure 1. Mechanistic action of enzymes selected for CLEA design: Endonuclease breaks the noncovalent interactions in the amorphous structure of cellulose. Exocellulase hydrolyses chain ends to break the polymer into disaccharides. Cellobiase hydrolyses disaccharides into glucose and finally glucose oxidase converts glucose to gluconic acid as well as hydrogen peroxide, leading to synergistic effects.

2.1 Immobilized cellulases

One approach to solve the above is to immobilize the cellulase to achieve controlled biostoning and minimal tensile strength loss. Numerous solutions have been implemented such as pumice immobilized cellulose [10], however, immobilization on solid support requires a good understanding of the support contribution to the catalysis and interactions with the substrate [11, 13, 14]. Therefore, an alternative is to make clusters of cellulase mimicking bacterial cellulosomes [15]. Insoluble enzyme aggregates formed by extensive chemical cross-linking of enzymes dissolved in a solution already have been suggested as an alternative approach for obtaining stable, reusable and more robust biocatalysts preparations [16].

The fundamental idea of our attempt builds upon modifications of the standard CLEA preparation to provide a tailored solution to reactive dyes and contrast control in denim washing. Figure 2 illustrates the rationale of modified CLEA design for controlled biostoning.

There are numerous issues to be addressed before achieving such a goal. The following three issues are considered to be the most challenging ones that need to be solved in order to produce innovative surface modifications on textile materials:

(i) What mediates the controlled penetration of free/immobilized cellulase into cotton?

(ii) How do immobilized cellulases access to indigo molecules in a fabric?

(iii) What parameters are critical to extended operational stability (performance and recovery) of immobilized cellulase?

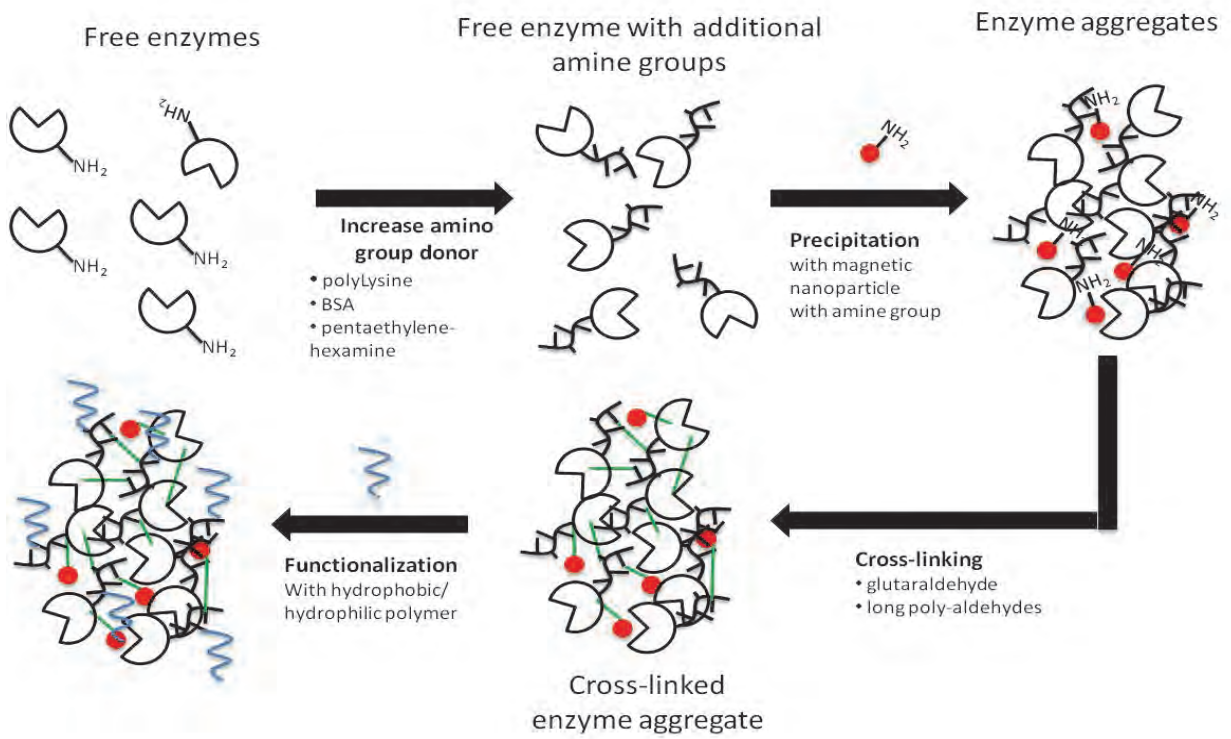

Figure 2. Concept with the individual steps for modified crosllinked enzyme aggregates (CLEA)

3. RESULTS AND DISCUSSION

Chemicals ammonium sulfate, glutaraldehyde, and Bovine Serum Albumin (BSA) were purchased from Sigma Aldrich and used as-purchased. The enzymes were purchased from Novozyme (Celluclast 1.5 L FG and Denimax ® Core 6400S) and Nuy Tekstil (Ecostone XC 300). Stock solutions of saturated ammonium sulfate (pH 7), Celluclast (20 mg/mL), BSA (100 mg/mL), glutaraldehyde (50%, w/w), magnetite solution (15 mg/L), Tris buffer (50 mM, pH 7.5), citric acid buffer (50 mM, pH 4.5 & 6.5) are utilized. Magnetic particles are prepared following a standard recipe published elsewhere [17, 18]. CLEA is prepared by a controlled precipitation, followed by crosslinking of enzymes. Validation of CLEA is based on the detection of reducing sugars released from the cellulose, estimated by 3,5-dinitrosalicylic acid (DNS). The released glucose was evaluated by Glucose Oxidase (GO) assay [19]. We focused on already identified cellulases for biostoning (Denimax ® Core 6400S and Ecostone XC 300) and a generic cellulase (Celluclast 1.5 L FG) for comparisons. The activity of the cellulases was primarily evaluated against filter paper (FP-cotton like substrate containing amorphous and crystalline fractions) and an amorphous soluble substrate (CMC carboxymethyl cellulose, predominantly amorphous). Table 1 summarizes the characteristics of the 3 selected enzymes.

Table 1. Comparison of three cellulases characteristics

Substrate	FP (cellulose)		Assay condition	Protein concentration	Ratio DNS/GO
	DNS [a]	GO [b]			
Celluclast	74 FP U/mL	10.4 U/mL	pH 5, 50°C	20 mg/mL	7.1
Ecostone [c]	17 FP U/g	2.4 U/g	pH 6.5, 50°C	50 mg/g	7.1
Denimax [c]	26 FP U/g	3.4 U/g	pH 6.5, 50°C	20 mg/g	7.7

[a] DNS method reflects the release of reducing sugars (mono and oligosaccharides)
[b] GO method reflects the level of glucose in the samples.
[c] Solid granules

For a rapid understanding of the enzymes' effects and translation of the assay to CLEA evaluation, two detection methods were chosen: DNS and GO. The rationale behind is simply that DNS -although sensitive to reducing sugars-, will overestimate the glucose concentration from the thermal degradation of oligosaccharides into glucose. On the other hand, GO will only report on the true amount of glucose release by the enzymatic hydrolysis. What we observe from the Table 1 is that DNS and GO within enzymes are different, which we attributed to the fact that oligosaccharides' thermal degradation yielded more glucose. Interestingly, the similarity between GO results accross the three enzymes suggests that all three have the same cellulase composition.

3.1 Enhancing cellulases abrasion capacity using pre-treatment and its recovery

In nature, cellulosome complexes produced by many microorganisms are defined as intricate multi-enzyme machines with numerous functional domains interacting with each other as well as with the cellulosic substrate in an *orchestral manner* to achieve the full capacity. More specifically, in the presence of denim as a substrate, the hydrophobic patchiness of the cellulases plays a key role [20-23]. Based on this hypothesis, we explored the role of BSA as an enhancing factor for biostoning but also as filler for CLEA formation. We demonstrated (data not shown) that cellulase biostoning can be enhanced by utilizing the weak affinity of BSA for cellulose and the opposite for cellulose/indigo. This remarkable result conforted us in the usage of BSA as both a filler for CLEA formation but also as an enhancing factor to facilitate cellulase biostoning. In addition to the standard CLEA preparation, we have been able to modify the CLEA design with magnetic Fe_3O_4 nano-particles to induce fast recovery from a turbid suspension as shown in Figure 3.

4. CONCLUSIONS

We have shown a design concept for CLEA as a cellulosome mimic with application to biostoning processes. Our experiments show encouraging results for the understanding of protein pre-treatment effects and successful magnetic CLEA fabrication.

Our preliminary results, representing both the activity of CLEA and free enzymes against FP as well as CMC as substrates, are shown in Table 2. The heterogeneity introduced within the substrate, strongly influence the CLEA activity, especially in case of CMC where the results show almost similar activities for both systems. However, the FP activities are dramatically different. At this early stage of the development, we can only conclude that the difference comes from either a detrimental structural change in the cellulase thus impeding their activity, or a size mismatch between the CLEA and the FP accessibility. Therefore, our current effort is to find the governing rules behind the following variables: CLEA fabrication parameters, aggregate size and size distribution as well as the overall biostoning activity.

Table 2. Comparison of free enzyme and CLEA activities

Enzymes	Filter Paper (FP)	Carboxy-Methyl Cellulose (CMC)
Celluclast	74 U/mL	98 U/mL
Celluclast CLEA	12 U/mL	72 U/mL

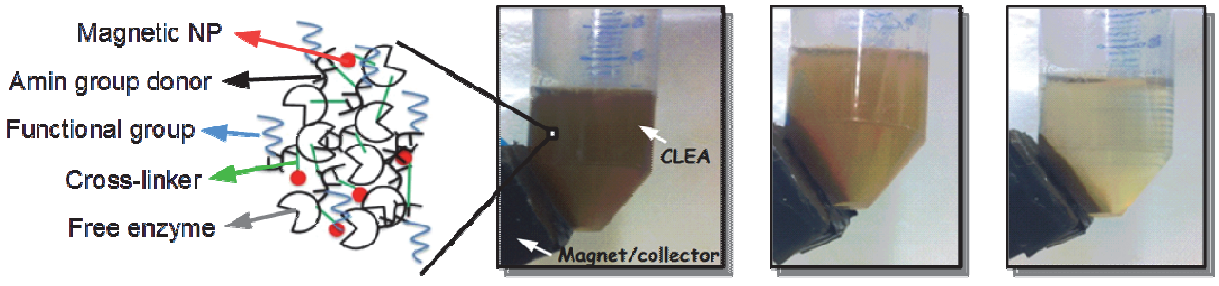

Figure 3. Recovery of magnetic CLEA using a magnet. From left to right the CLEA are progressively attracted to the magnet, and eventually a clear suspension is obtained.

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