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Application of Immobilized Glucose Oxidase in Iron Oxide Nanoparticles in Cotton Bleaching – An Ecofriendly Approach and Greener Alternative

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Abstract**—** Cotton, a natural textile fiber that naturally exists with impurities and yellowish color until bleaching that imparts whiteness to the fabrics. In this current research, iron oxide nanoparticles were synthesized and immobilized with microbially produced glucose oxidase which visualized by scanning electron microscope showed 62 and 112 nm respectively. Conventional and bio-bleaching of desized fabrics was done efficiently. Weight loss and water absorbency results of bio-bleached fabrics had shown higher effect than conventional whereas vice-versa in tensile strength. To ensure the quality of bleaching, whiteness index was performed and showed 65.21, 67.14 and 69.12 that corresponds for conventional and bio-bleached fabrics. Recyclability of immobilized glucose oxidase enzyme was showed better usage for 2 cycles which were enclosed by the comparison of whiteness index. Hence, we conclude that immobilized enzymatic bleaching showed significance which lacks in control and free enzyme treated fabrics.

Keywords**—** Bleaching, Glucose oxidase, Iron oxide nanoparticles, Whiteness index, Recyclability

I. Introduction

Textile industry plays a distinctive role as a fully integrated industry, from the creation of raw resources to the distribution of finished products, with considerable value incorporated at each phase of operation. Cotton fibers are the most favorite natural textile fiber due to their thermo-physiological comfort because of its desirable water absorption ability but the existence of natural grease wax and other impurities on their primary wall causes impenetrability that makes cotton fibers hydrophobic until undergone scouring and the degree of whiteness imparts by bleaching pretreatment [1]. One of the segments of the textile industry which holds a major share in the global pollution is wet processing sector [2]. Emerging of industrialization eventually imparts the textile sector towards 'EEE' process that is Ecological, Economical and Endure processes [3].

In the present scenario, clean production approach includes process optimization, free of chemicals, implementation of newer technologies, re-use of water would be the hopeful solution for current pollution [4]. In cotton wet processing, traditional bleaching agents like sodium hydrosulfite, hydrogen peroxide, peracetic acid have some drawbacks includes consumption of an enormous quantity of water and wastewater generation with chemical residues. In order to overcome this problem, enzyme technology, actually comprises unique production and its extensive applications in textile and fashion sector that drive the future [3,5]. Enzymatic treatments are virtually applicable because they are specific, eco-friendly and non-toxic as well as energy conservative. It is pertinent to mention that oxidase enzymes still have not been usually reported, would show a noteworthy result in cotton pretreatments equal to highly mentioned hydrolytic enzymes. Commercially available hydrogen peroxide could be generated by glucose oxidase enzyme by oxidizing glucose that aids in bleaching of cotton fabrics that would get rid of detrimental effects caused by traditional chemicals usage.

Since the enzymes are a precious gift for us from nature, they are not consumed for reactions thus raise the cost. Hence, there is cut-throat competition amongst the textile industries which necessitates bringing down the cost of production [6]. Knowing that it is difficult to strain the enzyme for reuse, immobilization of enzyme onto iron oxide nanoparticles would render the help for reuse further. Manufacturing of fabrics with newer technologies and environmental issues are two important aspects in the textile industry that has increased the awareness among researchers to include biotechnology as a promising solution. Indeed, the hotheaded growth of nanotechnology was achieved by boosted innovations in multiple areas. Iron oxide nanoparticles now able to revolutionize the textile field next to medical due to its hardcore stable and magnetic properties [7].

Implementation of enzymes in textile industry would play an efficient role as such chemicals do in addition with more significant impacts includes enrich the environment as well as enlighten the quality of fabrics.

II. RELATED WORK

Based on the abovementioned aspects, the potential present research involved in development of enzymatic bleaching using immobilized glucose oxidase on iron oxide nanoparticles rather traditional and their significance in reusability lacks in native enzymes and this aspect was not found in literature up-to-date

III. METHODOLOGY

Glucose oxidase enzyme and all the chemicals needed were purchased commercially from Hi-Media chemicals private limited, Mumbai, India. Desized cotton fabric sample was obtained from small-scale Textile industry, Tirupur, Tamilnadu, India.

Synthesis of iron oxide nanoparticles

Iron solution using ferric chloride and ferrous sulphate was prepared and added to sodium hydroxide solution under magnetic stirring which heated previously at 75ºC in the water bath. Formation of black precipitate would occur that indicated the presence of iron oxide nanoparticles having to pull attention to external magnetic force. Then, the solution was centrifuged at 5000 rpm for 15 mins after stirred vigorously for 30 minutes followed by decantation of the supernatant. At the point, the particles were washed several times with deionized water, shadowed by centrifugation at 3000 rpm for 10 minutes. Iron oxide nanoparticles were then subjected to lyophilization and stored after dried at 180ºC in an oven [8,9]

Immobilization of enzymes on the iron oxide nanoparticles

The adsorption method was used to immobilize glucose oxidase on the synthesized iron oxide nanoparticles. For this purpose, activated iron oxide nanoparticles by 10% glutaraldehyde were dispersed in enzyme solution (180 U/ml) and shaken in a shaker-incubator. A magnetic field was used to separate the magnetite from the solution and then it was washed with acetate buffer solution to eliminate the excess of glucose oxidase [10].

Confirmation of enzyme immobilization on nanoparticles by size using HRSEM analysis

High-Resolution Scanning Electron Microscopy (HRSEM) analysis was done to enzyme immobilized iron oxide nanoparticles and enzyme-free nanoparticles that identify the surface morphology and the size of the nanoparticles. In addition to that size of nanoparticles reveals the differences between them which also help us to conclude the immobilization of enzyme was done efficiently. Thin film specimens were attained by spreading it on dual side carbon tape and visualized under a scanning electron microscope. The descriptions of iron oxide nanoparticles were pictured and the morphology was determined [11].

Conventional bleaching of cotton fabrics

The desized cotton fabric was conventionally bleached with 3.5% of sodium silicate o.w.f. (the weight of fabric), 1% sodium carbonate (o.w.f)., 1% sodium hydroxide (o.w.f) and 4% of 35% hydrogen peroxide (o.w.f) for 1 hr at 90°C [12].

Biobleaching of cotton fabrics

Bleaching bath was prepared by addition of 1% glucose, 1% glucose oxidase enzyme, 0.4% magnesium sulfate, 1% Triton X 100 and treated at 90°C for 1 hr. The enzyme treated samples were taken out of the water and put into quenching water bath at 100°C for 10 minutes to deactivate the enzymes. The fabrics were then washed several times with hot water subsequently with cold water and dried at 100°C.

Immobilized enzyme bleaching

Immobilized enzymatic bleaching was done as per previously mentioned protocol by addition of immobilized enzyme instead of free enzyme.

Reuse of immobilized enzymes

The reusability of the immobilized enzyme was studied by repeated usage for approximately 5 to 10 times. In each cycle, the recollected enzyme immobilized nanoparticles were checked for its activity. The activity determined the first time was considered 100% for the calculation of remaining percentage activity after each use [13].

Assessment of physical properties of bleached fabrics

Weight loss method

The change in the basis weight of each fabric reflects the degree of hydrolysis that has occurred during the treatment of fabrics. The weight loss of fabrics was measured before and after treatment. Before and after treatment, the sample was weighed and the values were noted [11]. This test was done in triplicate and the average was calculated. Weight loss was calculated by using the following formula:

$$\% \text{ Weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Evaluation of wettability (AATCC 39 - 1980)

According to an AATCC Test Method 39-1980, water absorbency was monitored by placing a drop of water on the fabric where the droplet took time to enter inside the fabric was noted regarded as wetting time. The rate of fabric absorbency is indirectly proportional to the wetting time. This test was done in triplicate and the average was calculated [14].

Tensile strength measurement using Strip test

The tensile strength of the fabric samples was measured using tensile strength tester according to ASTM D5034-08. The sample strip size of 180 mm x 50 mm was precisely cut and the analysis was done at 50mm/min stretching rate. This test was done in triplicate and the average was calculated [15].

Whiteness of bleached fabrics

The fabrics were measured for their whiteness index in a spectrophotometer at 330 – 700 nm using Mac Beth 7000A spectrophotometer. This test was done in triplicate and the average was calculated.

IV. Results and Discussion

In this work, iron oxide nanoparticles was chemically synthesized using co-precipitation method was prepared by dropping iron solution into a basic solution where noticed a black color precipitate formation on further mixing which indicated the presence of iron oxide nanoparticles that confirmed by attraction towards external magnetic field and washed with water and ethanol consequently for excess reagent removal that has been portrayed in Figure 1. According to Rajesh *et al*., [11] the formation of the black color in the basic solution indicated the formation of iron oxide nanoparticles. In industrial scale, enzyme immobilization is a key factor that would stabilize the enzyme performance. Advances of enzymology and nanotechnology, nanoparticles have gained an interest as novel solid support because of their unique physicochemical properties. The nanomaterial possesses ideal characteristics to equilibrate principal factors which determine biocatalysts efficiency, including specific surface area, mass transfer resistance, and effective enzyme loading. As per author Ahmad and Sardar [16] statement referred stiffness, elasticity and biocompatible environments was strongly influenced for enzyme immobilization onto iron oxide nanoparticles. Thus, under this application of immobilization the enzyme glucose oxidase is immobilized covalently to the iron oxide nanoparticle.

Ahmad and Sardar, [16] studies undoubtedly showed the enzymatic immobilization on other metal nanoparticles has been studied using glucose oxidase enzyme reporting different applications. A literature review showed the diverse studies in iron nanoparticle synthesis revealed the same as the previous author's report. Immobilization of the glucose oxidase enzyme was done with the activated nanoparticles with glutaraldehyde was found to be the most frequently used for cross-linking purpose in many studies. Functionalization of iron oxide nanoparticles with glutaraldehyde might play a crucial role in binding of an enzyme to the support.

High-resolution scanning electron microscopy analysis

Confirmation of enzyme immobilization onto iron nanoparticles based on size was done with scanning electron microscopy and also it was employed in order to identify the surface morphology of the nanoparticles. Surface morphology and the size of control and enzyme immobilized nanoparticles were determined as illustrated in Figure 2 that represents spherical nanoparticles were found in clusters and sizes ranging from 62 nm to 92 nm. Nanoparticles merely not found an individual in the solution because of ferric chloride ions that acquired strong magnetic property towards each other thereby size difference.

According to Figure 2, we obviously found out enzyme immobilized iron oxide nanoparticles were appeared larger having higher size and vice-versa for enzyme-free nanoparticles indicated by Mohammed *et al*., 2017 in their research findings. Meanwhile, the research findings of Lassoued *et al*., [17] clearly defined that diameter of average nanoparticles was increased from 21 nm to 82 nm and difference in the size of nanoparticles would the presence of ferric chloride ions.

People have chosen to wear out cotton fabrics comfortably among other fabrics owing to its splendid properties and high moisture absorbency. Natural impurities and sizing materials were removed by desizing and scouring followed by bleaching which imparts whiteness of fabrics together referred to as wet processing technologies. Even though, hydrogen peroxide was found to be an ideal choice for effective bleaching for several decades, releasing of peroxide residues in waste-water and high consumption of water in rinsing step would have great concern among several researchers. In order to achieve whiteness of woven cotton fabric, enzymatic bleaching was designed in this study. As a first process, Sizing agents were removed using amylase enzyme currently employed for desizing in entire textile industries in the grey woven fabric and it was then exposed for whitening.

Bleaching plays a key role in providing the degree of whiteness to the fabric. Conventional bleaching was performed with commercially available hydrogen peroxide as per previously mentioned method. Conferring to the Ardon *et al*., [18] statement, the main objective of bleaching was to a removal of natural pigmented materials that are present in the fabrics eventually results in white appearance. The whiteness of the fabrics needs to be achieved without any losses in fabric strength. Peroxide residues were removed by a huge quantity of water which washes the fabrics.

Enzymatic bleaching would trend back in upcoming years because of its signifying aspects at treatment time, usage of chemicals, reduced oxygen demand level in the textile effluent compared with previously reported ones. Moreover, it greatly enhances the dye utilization of bleached cotton fabric while dyeing. After chemical bleach was over, enzymatic and immobilized enzymatic bleaching with glucose oxidase was done to the fabrics. The efficiency of the bleaching was detected by the values of various physical tests of the fabric relatively showed below. By these methods, the present result showed here confirms the greater performance by enzymatic bleaching in comparison with a conventional agent that is in line with the findings of Farooq *et al*., [19] who harvests the good quality results for enzymatic bleaching rather conventional.

Reusability of the immobilized enzyme is one of the major aspects of this present research. Investigation of recyclability property was done by continuously exposing the new fabrics to bleach until the enzyme loses its activity. Enzyme activity was measured after each bleaching process was over. Table 1 illustrated that the reusability efficiency of immobilized glucose oxidase on nanoparticles was lessened after the second cycle and their enzyme activity was found to be 34% of the initial value. It has been noted that the whiteness index and bursting strength of the treated fabrics while recycling was found to be 62.23 and 54.14, 4 and 3.2 respectively for the first cycle and second cycles. As noted on earlier reports, enzyme loss might be happened due to denaturation, inhibition of product formation or modification of the enzyme structure.

Investigation of physical properties of bleached cotton fabrics was mandatory to ensure enriched excellence results, weight loss, water absorption; tensile strength and degree of whiteness were measured according to standard methods. In weight loss, conventionally and enzyme bleached fabrics were calculated in terms of percentage. The significant difference between the initial and final weight of the bleached fabric sample after the treatment was regarded as weight loss. The results of weight loss of bleached samples are recorded and the higher impurities removal was found in all the fabrics which were bleached. The conventionally bleached, enzyme and immobilized enzyme bleached fabrics were found to have a weight loss of 1.19%, 1.22%, and 1.23%, respectively were demonstrated in Figure 3. All bleached fabrics were found to have almost equal weight loss but it was a little bit higher in enzyme bleached fabrics when compared to conventional one. Hence, it merely indicated that enzymatic bleaching is good as traditional bleaching. Paulo and Almeida *et al*., [20] conveyed that weight loss occurring during the treatments was accounted for an unsolvable deposit of microfibrillar cotton debris present in the fabric samples. Result for both absorbency and total weight loss of the fabric was directly proportional to each other.

Water absorbency of unbleached, conventionally bleached and enzyme bleached fabrics was calculated in terms of seconds. The results for all samples were done and it was understood that the samples showed a significant difference in absorbency properties. Enzyme treated fabrics have shown lesser time to absorb water was found to be 1 sec merely equal in both immobilized enzyme and conventional bleached fabrics which required one more second to absorb the water in the fabric. The unbleached fabric was used as a control where it requires more than 30 seconds were illustrated in Figure 4. This clearly suggested that enzyme-treated fabrics had shown lessened time to highest water absorbing capacity when compared to conventional and unbleached fabrics which were expected after getting the results for weight loss since both the results are directly proportional to each other.

A tensile strength of the fabrics will always show a substantial role in the fabric quality. The tensile strength of the bleached and unbleached fabrics was presented in Figure 5. The tensile strength of the untreated fabric, conventionally treated, enzyme treated, and immobilized enzyme treated bleached fabrics were documented as 52, 45, 41 and 42 lbs in the warp direction and 45, 37, 35 and 35 lbs in weft direction respectively that was revealed from Figure 5. The result has shown that the maximum tensile strength was documented for conventional bleaching rather than enzymatic bleaching where the weft-wise tensile strength of the fabric has lesser strength than that of the warp-wise tensile strength of the fabric. This was happening because of implying sizing material on the warm side during the weaving process while weft yarn has no sizing material.

Thus, it clearly proved that the tensile strength of the enzyme bleached was slightly lower when compared to the conventionally bleached. Moreover, the result for both tensile strength and total weight loss of the fabric was indirectly proportional to each other. Higher in weight loss, lower in tensile strength was proved from above said results. Ramadan, [21] had been reported that the tensile strength of the biobleaching fabrics has higher values than that of conventional bleached ones nearly about 21 and 17 kg respectively that was similar to the results obtained.

Whiteness index of treated fabrics

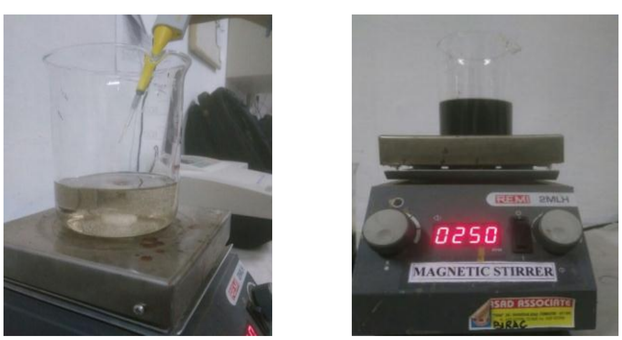
Bleached fabrics were evaluated for its whiteness using Macbeth spectrophotometer before imparting the color. The whiteness of the treated fabrics was expressed in Whiteness Index (CIE- D65) and the results are plotted in Figure 6. Removal of natural pigments in cotton fiber is achieved during the scouring step, some of them could not be removed and adsorbed to the persisted pectic and protein substances in the cotton fiber. Thus, some increase in whiteness index values was noted upon the removal of these pectic and protein substances from cotton fiber. The whiteness index values of untreated grey fabrics, conventional bleaching, biobleaching, and immobilized biobleaching treated fabrics were found to be 13.20, 65.21, 67.14 and 69.12. The results show that the untreated and treated fabrics have maximum whiteness indexes.

Wasif and Indi, [22] declared in their statement that coloration and fabric finishing was done only after bleaching would found as the key aspect for a better quality enrichment of the fabrics. Several research findings have and have not been shown that the whiteness was increased for enzymatic bleaching. From the results, in enzymatic bleaching, we conclude that it is possible to bleach efficiently if it was done ineffectively in the optimized manner that eventually increases the degree of whiteness when compared to traditional one. It also suggested that minimum time exposure would give a better result if prolonged; the value of the whiteness was decreased. The minimum concentration of the glucose oxidase eventually results in stable hydrogen peroxide production, thus aids in effective bleaching. As per Anusuya and Srimathi's statement [23] and the present research findings, it was proved that enzymes found to be good alternative and great usage in the field of textiles.

V. CONCLUSION

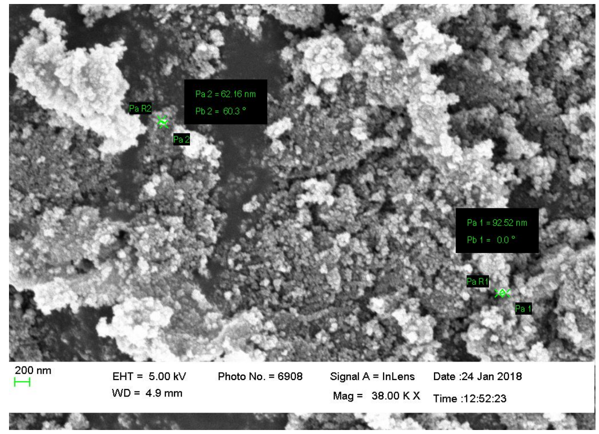
Established biotechnological processes that are currently in use demonstrate the potential of industrial biotechnology to successfully provide greener alternatives. All of the above results showed promising prospects of using the immobilized enzyme in textile bleaching with reusability property. In the present scenario, the quality control in processing is becoming more important besides cost control. Therefore, in addition to energy saving benefits, there is the emphasis on prediction of quality parameters like whiteness index, strength loss, and weight loss of fabrics. Biotechnology is making the significant contribution in enhancing the eco-efficiency of textile processing sectors and improvement in the quality of human life for the upcoming decades. Further intensified research on enhancing the efficiency of enzyme processes, methods for reuse of enzymes in more number of cycles will be considered for continuous improvement to overcome obstacles in cost-wise by textile processing sector in an ever-evolving textile industry.

Figures and Tables

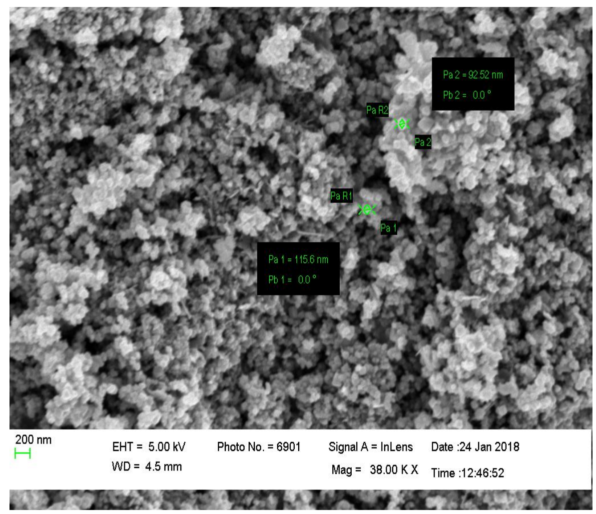


a. Dropping of iron solution b. Formation of iron nanoparticles

Figure 1: synthesis of iron oxide nanoparticles



a. Control INP



b. Enzyme immobilized INP

Fig 2 Scanning electron microscope image of INP and enzyme immobilized INP

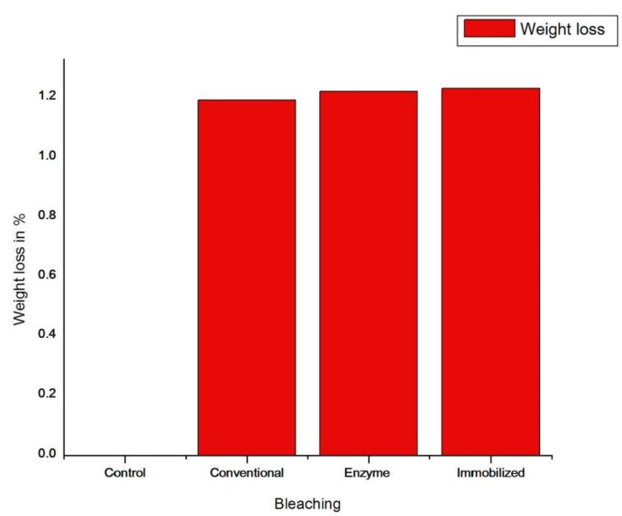


Figure 3 Weight loss of bleached fabrics

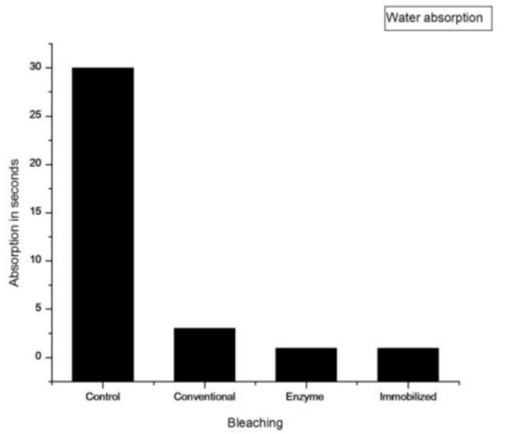


Figure 4 Water absorbency of bleached fabrics

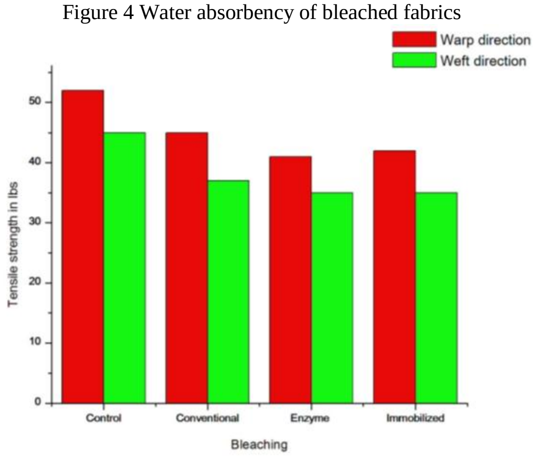


Figure 5 Tensile strength of bleached fabrics

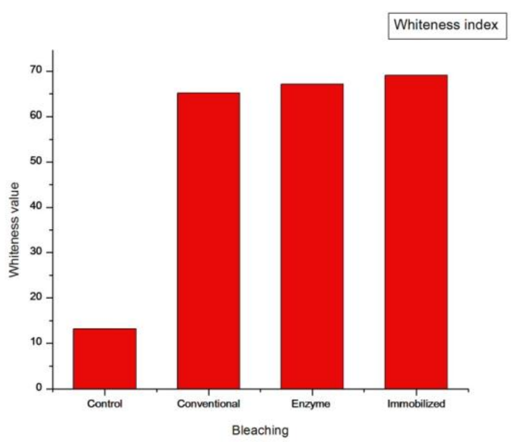


Figure 5 Whiteness index of bleached fabrics

Table 1 – Reusability of immobilized enzyme

Bleaching cycles	Whiteness index	Bursting strength	Enzyme activity (%)
First	62.23	4	100
Second	54.14	3.2	69.2
Third	37.23	2.4	34

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