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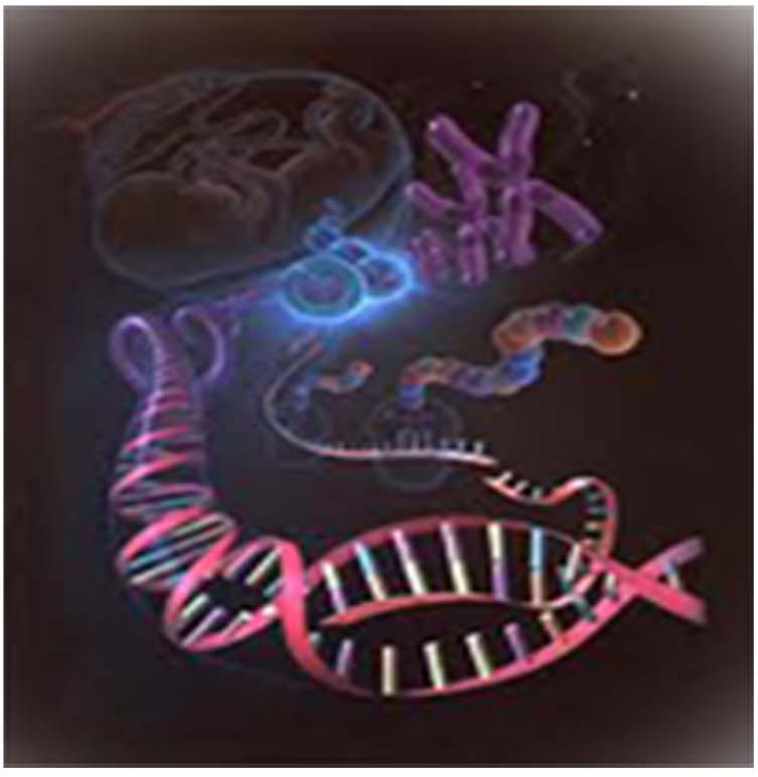

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Research Paper

IMMOBILIZATION OF *VIGNA RADIATA*, *VIGNA MUNGO*, *CICER ARIETINUM* (WHITE) AND *CICER ARIETINUM* (BLACK) AMYLASES ONTO VARIETY OF ACTIVATED FABRICS

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There has been considerable interest in the development of carrier systems for enzyme immobilization because immobilized enzymes have enhanced stability compared to free enzymes. This leads to significant savings in terms of reduced enzyme consumption and the ability to reuse of immobilized enzymes in continuous processes. The activity and stability of enzymes depends largely on the particular operating and storage conditions, and is strongly influenced by factors such as the chemical environment, temperature, pH, and solvent properties. This work was included the immobilization of *Vigna radiata, Vigna mungo, Cicer arietinum* (white) and *Cicer arietinum* (black) amylases onto variety of activated fabrics treated with sodium chloride and sodium nitrate. Comparative study was done for their % immobilization of retention of activity of pulses amylases on to the variety of activated fabrics after immobilization.

Keywords: *Vigna Radiata, Vigna Mungo, Cicer Arietinum* (white), *Cicer Arietinum* (black), silk, nylon, cotton, polyester

INTRODUCTION

Amylases are starch degrading enzymes. They are widely distributed in microbial, plant and animal kingdoms They are involved in the hydrolysis of starch molecules (Paquet *et al.*,1991). They are involved in the hydrolysis of starch molecules (Paquet *et al*., 1991), decomposing them in glucose. They degrade starch and related polymers to yield products characteristic of individual amylolytic enzymes. Initially the term amylase was used originally to designate enzymes capable of hydrolyzing α-1,4-glucosidic bonds of amylose, amylopectin, glycogen and their degradation products (Bernfeld, 1955; Fisher and Stein, 1960; and Myrback and Neumuller, 1950). They act by hydrolyzing bonds between adjacent glucose units, yielding products characteristic of the

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particular enzyme involved. In recent years a number of new enzymes associated with degradation of starch and related polysaccharides structures have been detected and studied (Buonocore *et al*., 1976; and Griffin and Fogarty, 1973). Amylases are classified into three viz., α-amylase, β-amylase and γ-amylase. Unlike β-amylase, α- and γ-amylases are produced in animal system abundantly, and all these three enzymes are produced by plants, yeast, fungi and bacteria. Four groups of starch converting enzymes have been identified viz., endoamylases, endo-amylases cleave α-1,4glycosidic bond in a random fashion present in the amylose or amylopectin chain (structural components of starch made of sugars) and β-amylase is a well known endoamylase.

Exoamylases such as β-amylase and glucoamylase cleave both α-1,4, and/or α-1,6 glycosidic bond, exoamylases, debranching enzymes and transferases. α-Amylase (alpha→1-4-glucan 4-glucanhydrolase, EC 3.2.1.1) is one of the key enzymes hydrolysing reserve starch in the endosperm of germinating cereals. It is synthesized *de novo* in the aleurone cells, in response to gibberellic acid (Yomo and Varner, 1971). Another form of amylase, β-amylase (EC3.2.1.2) (alternative names: 1,4-α-D-glucanmaltohydrolase; glycogenase; saccharogen amylase) is also synthesized by bacteria, fungi, and plants. Working from the non-reducing end, β-amylase catalyzes the hydrolysis of the second α-1,4glycosidic bond, cleaving off two glucose units (maltose) at a time.

During the ripening of fruit, α-amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit. γ-Amylase (EC3.2.1.3) (alternative names: Glucan 1,4-α-glucosidase; amylogluco-sidase; Exo-1,4-α-glucosidase; glucoamylase; lysosomal α-glucosidase; 1,4-α-D-glucangluco hydrolase) will cleave α (1-6) glycosidic linkages, as well as the last α(1-4) glycosidic linkages at the nonreducing end of amylose and amylopectin, yielding glucose. Unlike the other forms of amylase, α-amylase is most efficient in acidic environments and has an optimum pH of 3. *Vigna radiata* is an exalbuminous legume, in which the cotyledonary cells are filled with starchgrains as the main energy storage component. It is an annual food legume belonging to the subgenus *Ceratotropis* in the genus *Vigna*. The genus *Vigna*, together with the closely related genus *Phaseolus*, forms a complex taxonomic group, so called *Phaseolus-Vigna* complex (Verdcourt, 1970). Marechal *et al*. (1978) followed Verdcourt and presented a monograph on the *Phaseolus-Vigna* complex.

Three botanical varieties were recognized in their monograph. *Vigna radiata* is cultivated form (mung bean), var.*sublobata* is the wild ancestral form of mung bean, and var.*setulosa* is also wild form which distribute in India, Indonesia, southern China. Chickpea (*Cicer arietinum* (black) L.) is the world's third most important pulse crop after common bean (*Phaseolus vulgaris*) and pea (*Pisum sativum*). It is grown mainly on the Indian subcontinent, but also in Western Asia, North Africa, the Mediterranean basin, the United States, Canada and Australia.

India is the world leader in chickpea (Bengal gram) production followed by Pakistan and Turkey. The semi-arid regions of India and the Mediterranean. Today*, Cicer arietinum (white)* is native to the Middle East and has been grown traditionally throughout *Cicer arietinum (white)* is the third most important pulse crop (after dry bean and pea) and makes up 20% of the worldpulse production. Major producers of chickpea include India, Pakistan, and Mexico. *Vigna Mungo is an* erect, suberect or trailing, densely hairy, annual herb. The tap root produces a branched root system with smooth, rounded nodules. It is nutritious and is recommended for diabetics, as

are other pulses. *Vigna mungo* is used in traditional Indian (Ayurveda) medicine. Pharmacologically, extracts have demonstrated immunostimulatoryactivity (Solanki and Jain, Immunostimolatory). *Vigna mungo* originated from central Asia and India from where it started being domesticated. It is now found in many tropical areas of Asia, Africa and Madagascar.

An enzyme is termed immobilized, if its mobility has been restricted by chemical means. Immobilization of enzymes refers to techniques which represent variety of advantages over free enzyme catalysis including increased stability of enzyme, easy recovery of enzyme, easy separation of reactant and product, repeated or continuous use of a single batch of enzyme (Raviyan *et al*., 2003) which will ultimately save the enzyme, labour and overhead costs (Gerhartz, 1990). Immobilized enzymes have been widely used for many years in different industrial processes. Usually, immobilization of enzymes is carried out by three principle means, matrix assisted entrapment of enzyme, adsorption on a solid support, ionic or covalent binding (Swaisgood,1985; and Zaborsky, 1973).

Entrapment is taken as the most preferable method because it prevents excessive loss of enzyme activity after immobilization, increases enzyme stability in microenvironment of matrix, protects enzyme from microbial contamination (Kennedy, 1993). Physical entrapment of α-amylase in calcium alginate beads has shown to a relatively easy, rapid and safe technique (Dey *et al*., 2003) in comparison with other immobilization methods. The method of immobilization should be like that the enzyme structure might have least conformational change as possible. The nature of the solid support or matrix plays an important role in retaining the actual confirmation and activity of enzyme in the processes that utilized immobilized biocatalysts. Our work was based on study of % of immobilization of variety of pulses on to variety of different activated fabrics which are easily commercial available in the market to increase the storage stability and reusability of industrial important amylases.

MATERIALS AND METHODS

Extraction Amylase from Pulses

Cotyledons from 3-day old seedlings from germinating seeds of *Vigna radiata, Vigna mungo*, *Cicer Arietinum* (white) and *Cicer arietinum* (black) were homogenized using mortar and pestle in 0.05M sodium phosphate buffer (pH 7.0). 4-6 ml of buffer was added per gram of fresh weight. Enzyme extract was filtered through two layers of cloth and centrifuged for 15min at 4°C. The supernatant was collected which contained crude enzyme and stored at 4°C.

Amylase Assay

Amylase was assayed according to the procedure followed by Bernfeld, 1955. The activity of extracellular amylase was estimated by determining the amount of reducing sugars released from starch. The sugars were quantified by the method of 3,5-dinitrosalicylicacid (DNS), according to Miller (1959). The starch solution was prepared from 1% (w/v) soluble starch in distilled water. 0.5 ml of the enzyme extracts were added to 1 ml of the starch solution and the mixture was incubated at 37ºC for 20 min. After that 2 ml of DNS was added to terminate the reaction and the reaction mixture was boiled at 100ºC for 5 minutes. The amount of reducing sugars in the final mixture was determined spectrophoto-metrically at 570 nm. One unit of

enzymatic activity is defined as the amount of enzyme that produces 1 µmol of maltose per minute (Robert and Evan, 2003; and Garen and Levinthal, 1960).

Chlorine and $NaNO_3$ Treatment of Fabrics

Bombyx mori silk, nylon, cotton and polyester fabrics were chlorinated with NaCl solution (cholrine content, 3%) and then treated according to the procedure reported previously (Furuhata *et al*.,1996; and Rani, 2012). Amylase was extracted and purified from *Vigna radiata* and *Cicer arietinum* (black). All chemicals used were of analytical grade. Another batch of the same fabrics was treated with $NaNO_3$ and the same procedure was followed.

Immobilization of Amylase onto Variety of Activated Fabrics

Pieces of treated fabrics (10-15 mg) were put into a flask and 5 ml solution of an amylase (1 mg/ml) was added. The flask was kept at 37°C for 24 hours with occasional stirring. After the treatment, the fabric pieces were taken out and the remaining solution was analyzed colorimetrically for the enzyme activity which was to be calculated from standard curve of maltose. The amount of amylase immobilized onto the fabrics was calculated from decrease in enzyme concentration. The treated fabric pieces were washed several times with 1 M KCL for 2 hours at 30°C under shaking in incubator. These were stored in a refrigerator below 5°C by immersing them in 0.1 M KCL solution.

% Immobilization

The enzyme bound to NaCl treated and $NaNO_3$ treated fabrics was estimated by determining the residual specific activity from solution of enzyme during immobilization.

$$\% \text{ Retention} = \frac{\text{Specific Activity of Immobilized Enzyme}}{\text{Specific Activity of Free Enzyme}} \times 100$$

RESULTS AND DISCUSSION

It was observed that the percentage immobilization observed in NaCl and $NaNO_3$ treated silk was the

Figure 1: Comparative Percentage Immobilization of *Vigna radiata* Amylase onto NaCl Treated Fabrics

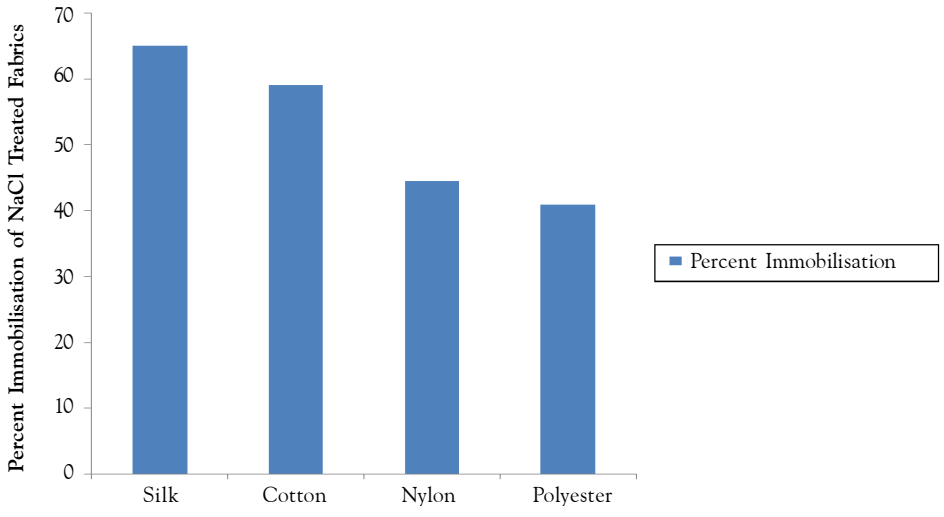
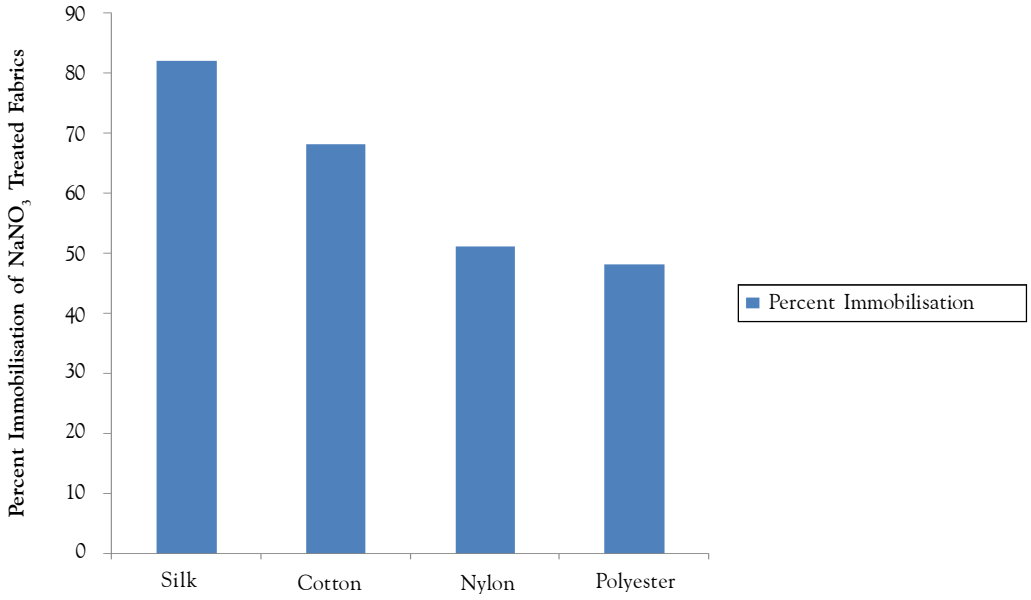


Figure 2: Comparative Percentage Immobilization of *Vigna radiata* Amylase onto $NaNO_3$ Treated Fabrics



highest in case of both *Vigna Radiata* and *Cicer Arietinum* (black) (Figures 1, 2, 3 and 4). After silk the immobilization was highest in cotton followed by polyester and nylon in case of *Vigna Radiata.* In *Cicer arietinum* (black) the highest percent immobilization was in silk followed by polyester, nylon and then cotton (Figure 3). It was also observed that treatment with $NaNO_3$ increased the percent immobilization. This was observed both in case of *Vigna radiata* and *Cicer*

Figure 3: Comparative Percentage Immobilization of *Cicer arietinum* (Black) Amylase onto NaCl Treated Fabrics

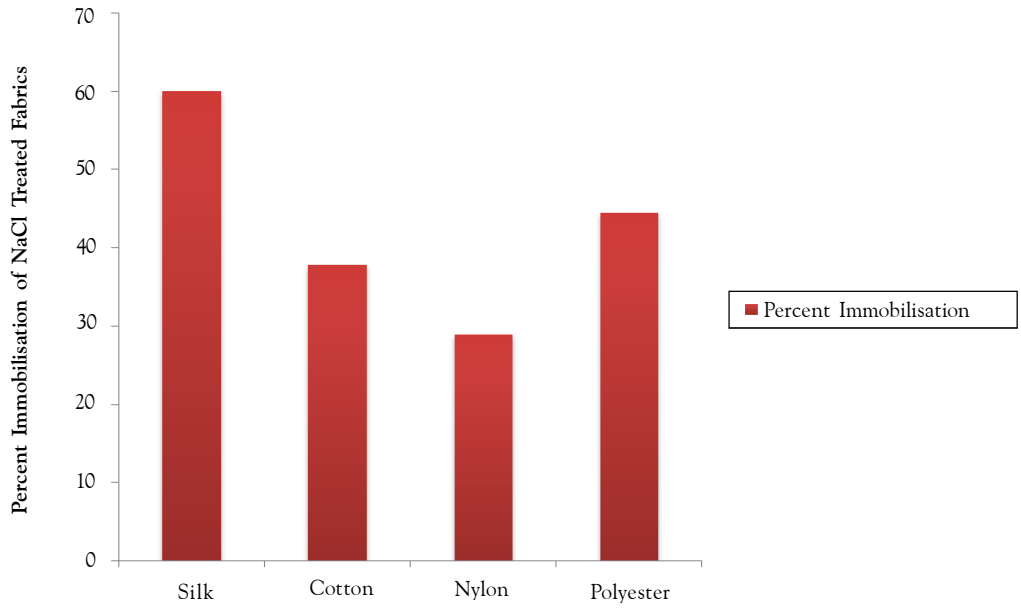


Figure 4: Comparative Percentage Immobilization of *Cicer arietinum* (Black) Amylase onto $NaNO_3$ Treated Fabrics

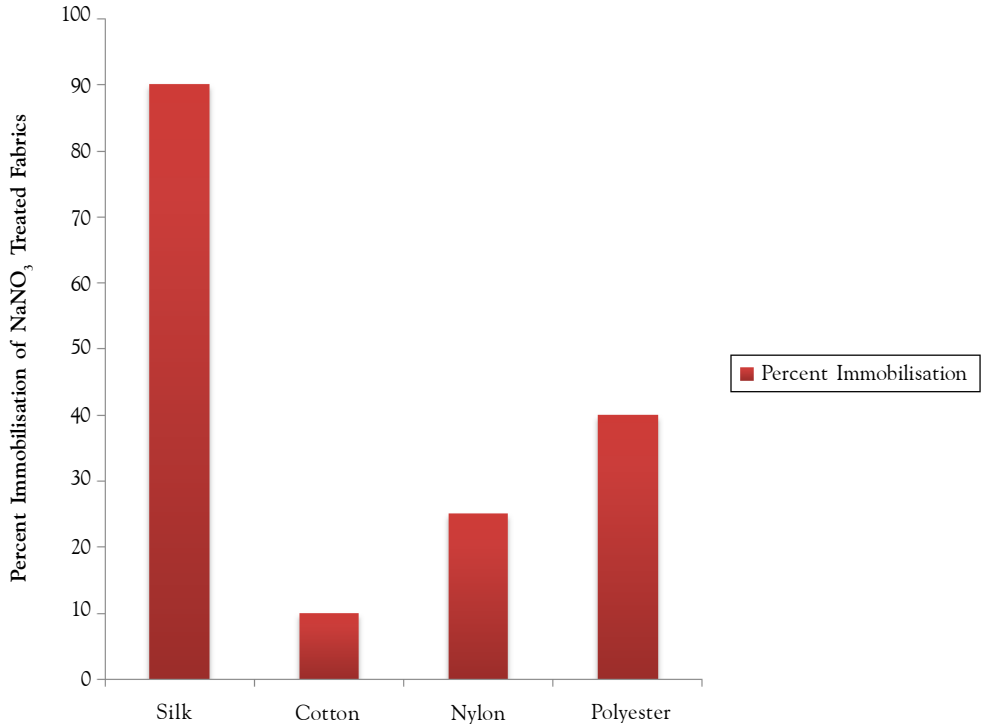


Figure 5: Percentage Immobilization of *Vigna mungo* Amylase onto NaCl Treated Fabrics

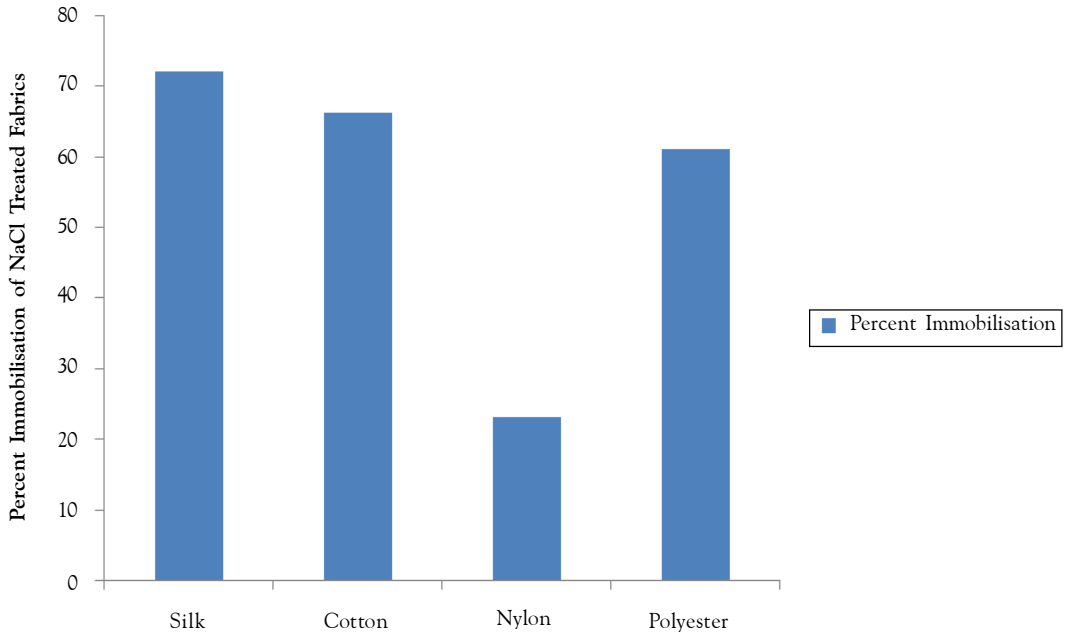


Figure 6: Percentage Immobilization of *Vigna mungo* Amylase onto NaNo3 Treated Fabrics

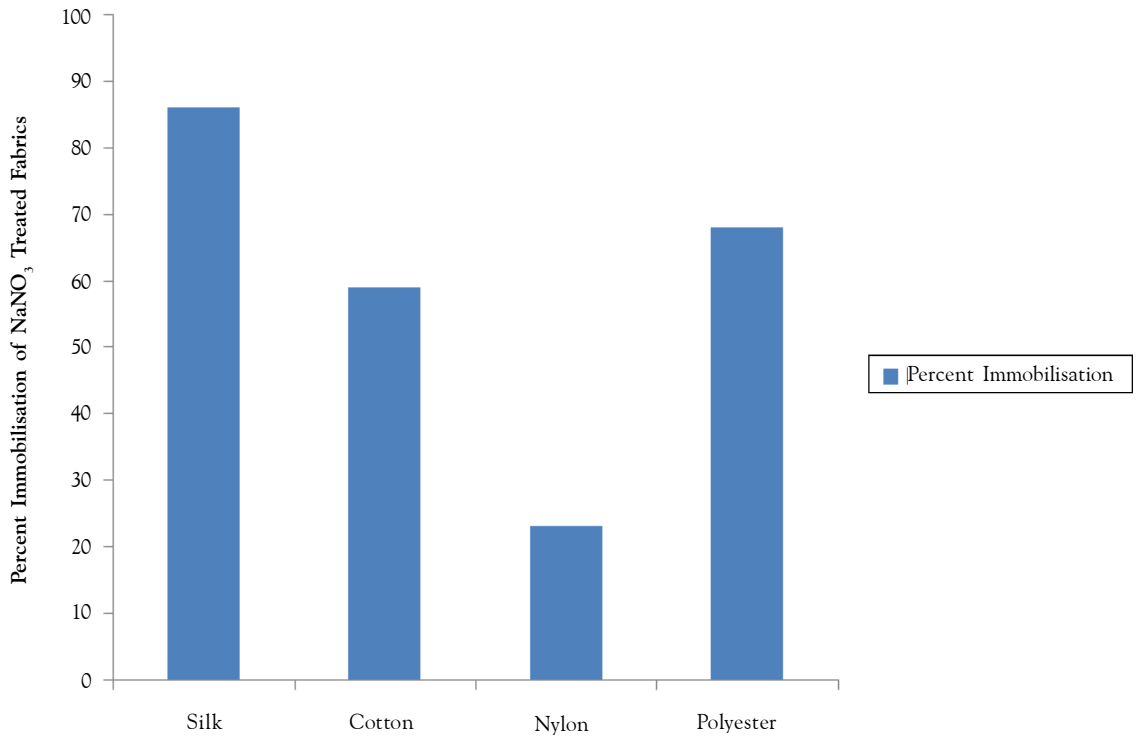


Figure 7: Percentage Immobilization of *Cicer arietinum* (White) Amylase onto NaCl Treated Fabrics

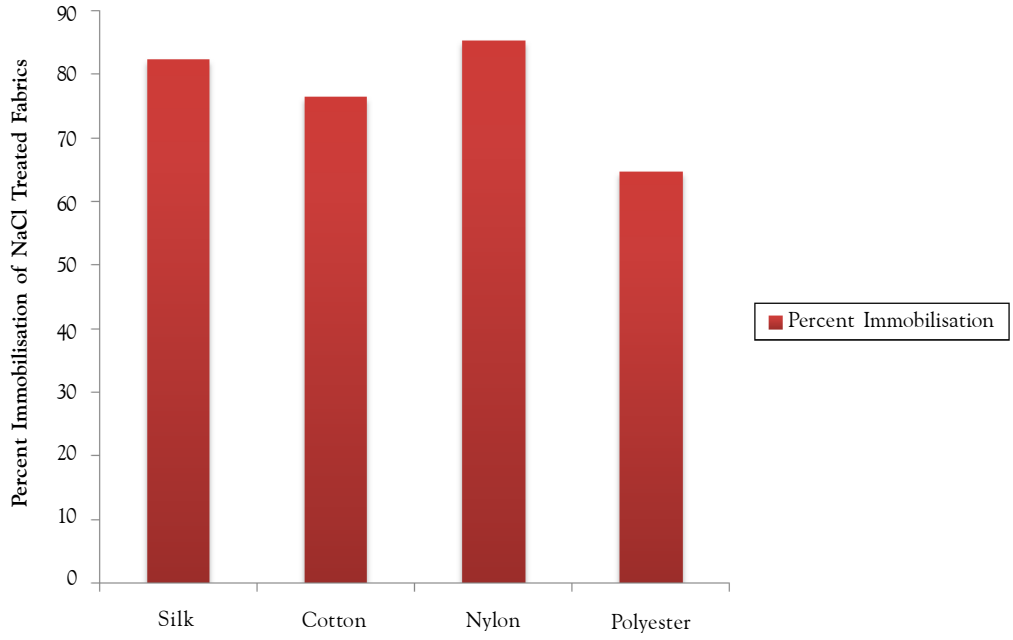
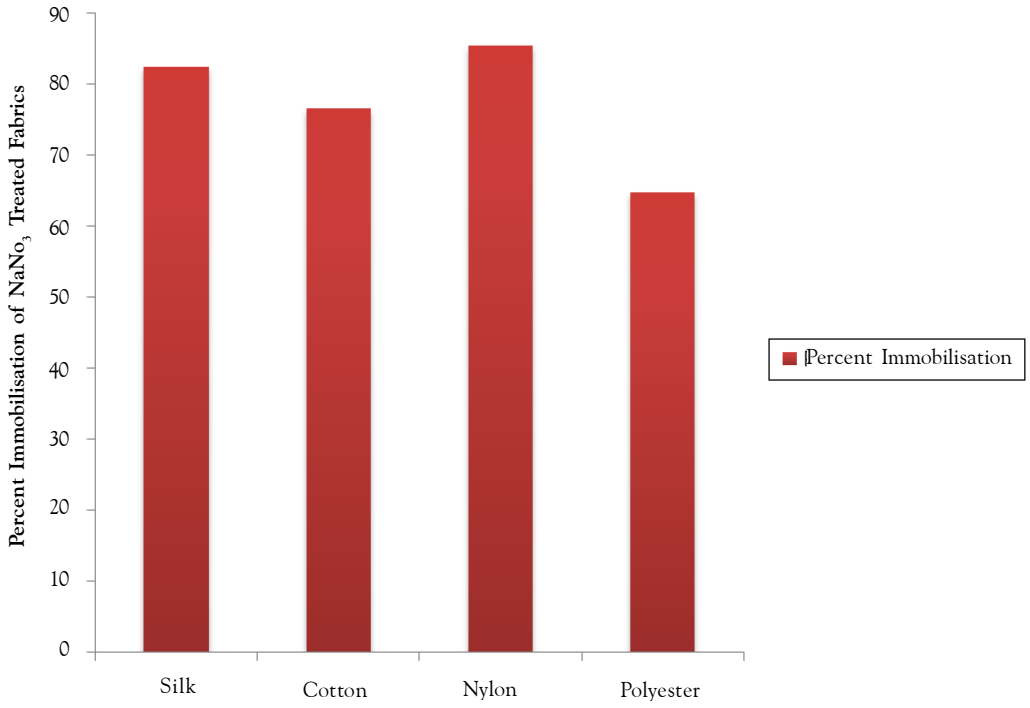


Figure 8: Percentage Immobilization of *Cicer arietinum* (White) Amylase onto $NaNo_3$ Treated Fabrics



arietinum (black) (Figures 2 and 4). From the above results, we conclude that the percentage of immobilization of *Vigna mungo* was highest on silk fabric in both NaCl treated as well as $NaNo_3$ treated fabrics (Figures 5 and 6). While in case of *Cicer arietinum* (white), the percentage of immobilization was maximum on nylon (Figures 7 and 8). Percentage of immobilization onto different activated fabrics was in the range of 70-99%. And after the immobilization, the pulses amylases were found stable for 3-4 months with the 40% of loss of their activity when stored at 4° C.

CONCLUSION

Thermostable amylase is one of the most important and widely used enzymes whose spectrum of application has widened in food, paper and detergent industries (Glazer *et al.*, 1994; and Nigam and Singh, 1995). These industries would find their boosted economy if amylase can be re-used which is possible by their immobilization. Industry demands an ideal amylase with increased stability, durability, reusability – especially when used at immobilised state, or a trifunctional amylase embodying the functions of all amylases with maximum activity. Such a versatile fusion protein should be useful in starch processing, starch liquefaction, fermentation, starch saccharification, washing, laundry, textile desizing, baking, and biofilm removal.

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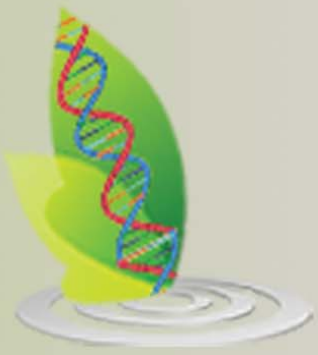

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