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Production, partial purification and immobilization of alpha amylase using new bacterial isolate and its nanoform application for modification of wool fibers dyed with madder natural dye

Ali, N. F.[1*] and Abd-El salam, I. S.[2,3]

[1]Dying and printing Department, Research and technology textile institute of textile industries, National research centre, Egypt; [2]Chemistry of natural and microbial products department, National research centre, Egypt; [3]Institute of Pharmaceutical industries and Drug Research National Research Center

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Abstract The amylase producing bacterial isolates *E coli* NRC1169 showed maximum amylase production and selected for physiological and biochemical reactions. Fermentation process on suitable medium showed the proper producing isolates. Partial purification of the crude enzyme, its characterization and using nano-chitosan as carrier were recorded. Partially purified enzyme was investigated in the field of textile for pre-treatment of wool fibres dyed with madder natural dye.-The maximum activity resulted in using fermentation medium consisted of starch ,10 peptone 10 yeast extract 5, ammonium sulphate 5, magnesium sulphate 0.25, CaCl 0.25 tween 80 1ml at pH 5.6, fermentation time 48 h at $30C^0$. The obtained amylase was undergoing partial purification using ammonium sulphate at concentration of 60%. The purified enzyme was applied to textile industries.–Result indicated that the wool fibres samples treated with enzyme free and immobilised exhibited higher colour strength and fastness properties than the untreated fibres. The antimicrobial activity were tested using *E. coli, B. subtilus, C. albicans, S. aureus* for the fibres treated with amylase gave higher inhibition than the untreated fibres.

Keyword: Amylase, Production, Purification, Immobilisation, Textile

Introduction

Natural dyes are non-polluting, regenerative, and good for the environment. Natural dyes are used to colour textile materials instead of synthetic dyes. They are not toxic or carcinogenic. Natural dyes are used to colour a variety of textile fabrics (Adeel *et al*., 2019). Natural colours are gentle on the skin, friendly to the environment, and pleasing to the eyes. Natural dyes are suitable for all sorts of materials used in businesses because of their distinct natural origin (Malacara, 2002). natural colours derived from various natural materials.

[*]**Corresponding Author:** Ali, N. F.; **Email:** aali_04@hotmail.com

A variety of textile materials are colored using natural dyes (Adeel *et al*., 2019). Natural hues are kind to the skin, eco-friendly, and aesthetically beautiful. Because of their particular natural origin, natural dyes are appropriate for all types of materials used in businesses (Malacara, 2002). All forms of materials utilized in industries, including natural hues obtained from a variety of natural materials (Malacara, 2002). natural dyes derived from various materials, such as plants and vegetables. They protect the body from UV radiation, have antimicrobial characteristics, and do not cause skin.

Natural dyes are coloring agents made from minerals, plants, insects, or vegetables. The use of natural dyes has become more popular as concerns about the environmental friendliness of various textile businesses have grown. There are more and more reports of natural dyes being used on textiles around the world. Numerous studies have also found that natural colors have therapeutic and antibacterial properties. According to research (Chequer *et al*., 2013; Samanta *et al*., 2008; and Shahmoradi Ghaheh *et al*., 2014).

As worries about the environmental friendliness of many textile industries have grown, the use of natural dyes has gained popularity. Natural dyes are being utilized on textiles all across the world, according to an increasing number of publications. Natural colors are also believed to offer healing and antimicrobial effects, according to numerous research. According to studies (Chequer *et al*., 2013; Samanta *et al*., 2008; and Shahmoradi Ghaheh *et al*., 2014), turmeric has been used as an antibacterial agent in medicine for a very long time because of its yellow hue.

It is interesting to note that a rise in the use of natural dyes has coincided with their increasing resuscitation phase and prevented the need for more chemicals in synthetic colors. It is likely due to people's worries about safety and pollution reduction. The demand for naturally colored organic fabrics has increased for prospective projects on the international market in recent years. This evaluation focuses on organically sourced natural dyes that are used to color wool fabric. The textile industry must strive on developing cutting-edge technology to reduce energy and water consumption. Microwaves offer the advantage over traditional methods in that they use less liquid, less color, and don't waste any liquid dye Madder is an important source of natural dye extracted from vegetables used for dyeing wool fabric. It is an old natural dye. It is named as the queen of red with the common name of Rubia Argyi that belongs to Rubiaceae (Ali and El-Khatib, 2016). The combined enzymatic processes technology used in the pre-treatment of textiles has become possibilities of enzymatic processes (Ali and Abd- El salam, 2020). It is suggested that using various enzymes namely amylase, protease, lipase, pectinase, laccase, glucose oxidase, catalase and cellulase from the beginning to the end of the preparatory processes of textile substrates could be achieved. The combined enzymatic

processes of textile materials were also achieved by several researchers (Atalla *et al*., 2019). The use of enzymes enhances the process efficiency, shortens the process time and requires the use of less energy, compared to the conventional methods which are carried out separately. In the Turkish textile companies, the enzymes namely amylase, pectinase and catalase are widely used in the preparatory processes.

A substantial starch processing sector has emerged during the past century. In recent years, starch-converting enzymes have replaced the acid hydrolysis of starch in the production of maltodextrin, modified starches, and glucose and fructose syrups. Approximately 30% of the world's total enzyme production is currently made up of these enzymes, according to van der Maarel *et al*. (2002). The goal was to look into how to increase amylase activity.

Material and methods

Microorganism

Escherichia coli, the bacterial isolate employed in this investigation, was chosen in accordance with initial screening studies of bacterial strains from soil sample. It was given the *E. coli* NRC1169 identification number and subjected to biochemical and genomic analysis in another research work. It was kept alive at $4C^0$ on a nutrient agar medium with subsequent monthly preculturing.

El Mahalla Company in Egypt provided the wool fibres 10/2. Madder dye was employed as a natural dye. Madder was utilized to produce the coloring material (Figure 1).

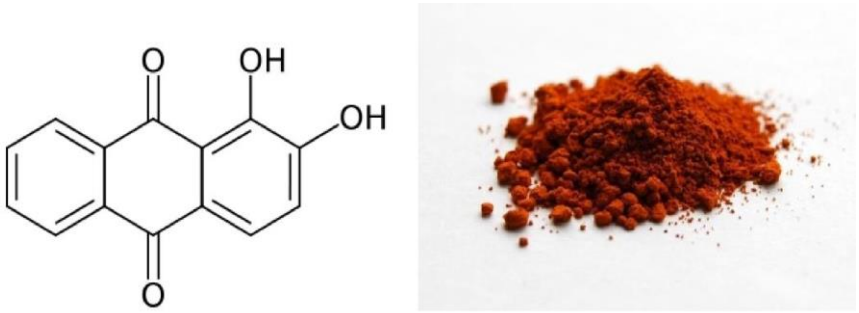

Figure 1: Chemical structure of Madder dye

Amylase synthesis

A prepared bacterium inoculum was added to 50 ml of nutrient broth, cultured at 30 C for 24 hours, and then centrifuged at 180 rpm. It

served as an inculation agent for the manufacturing medium used to make amylase. 250 ml Erlenmeyer flask containing sterilized 50 ml of the production medium, which was maintained at a pH of 6,3 and contained the following ingredients: g/l starch 10, peptone 10, yeast extract 20, manganese chloride 0.015, calcium chloride 0.05, potassium di hydrogen phosphate 0, magnesium sulphate 0.25, ferrous sulphate 0.01 and calcium chloride 0.015.48 hours of 48o rpm at 30C0 incubation was followed by enzyme estimation. According to Oyeleke *et al.* (2010), the amylase solution was created by centrifuging at 5000 rpm for 20 min.

Test for amylase

A test tube was filled with 1 ml of crude enzyme and 1 ml of 1% soluble starch in sodium phosphate buffer (pH 7). For 10 minutes, the test tubes were covered and heated to 35 ℃. After stopping the reaction with 2 ml of DNS reagent, each tube was placed in a pot of boiling water for 10 minutes. Using distilled water, the final volume was created to 10ml and let to cool at room temperature. Using maltose as a reference, the absorbance was measured using a spectrophotometer at 540 nm (Pokhrel *et al.*, 2013). One enzyme activity unit (U) was established as the quantity of enzyme that, under typical assay conditions, released 1 mol of glucose from the substrate in 1 minute.

Extraction of naturally occurring colourant

The madder sample was ground into a powder, and the colouring was extracted by boiling 20–80 g of the powder in 1000 ml of water for an hour while utilising conventional and microwave heating methods for the first five minutes. The solution was filtered and cooled after that using a microwave, different enzyme solution concentrations (5–20%) were applied to wool fibres for 5 minutes at a liquor ratio of 1:50%.

The dying process

Madder dye at various strengths of 20–80 g/L in a 1:100 liquor ratio is used in the dye bath. Wool cloth was coloured using a microwave at pH 5 for varying times ranging from 1 to 5 minutes. The samples that had been coloured which were rinsed in warm and cold water, washed in a bath of 5g/L non-ionic detergent that was heated to 50oC for 30 minutes, rinsed, and dried in air at room temperature.

Colour strength measurements (K/S value)

For the purpose of calculating the samples' reflectance, an UltraScan PRO spectrophotometer was employed. At a wavelength of 500 nm, the K/S

value was determined spectrophotometrically. Untreated and pre-treated wool fabrics with chitosan and tannic acid were assessed for their K/S values.

Fastness characteristics test

The dye samples were analysed according to ISO standard procedures after being rinsed off with 2 g/L nonionic detergent at 80 ℃ for 30 minutes. The specific tests, related to color fastness to rubbing, washing, perspiration, and light, were ISO 105-X12 (1987), ISO 105-C02 (1989), and ISO 105-B02 (1989). The samples' color variations were measured against a precise grey scale.

Results

Sscreening test and choosing a powerful isolate

One gram of soil sample was streaked on sterile petri dishes containing nutrient agar medium after being serially diluted. Resub cultures of the acquired colonies were performed using nutrient agar as the culture medium. 12 isolates were found to have amylase activity, according to the findings (Table 1). Analytical evaluation of amylase activity was only performed on the one active strain. The outcomes showed significant variations in amylase activity among the tested strains, with strain number 4 having the highest activity (189.51u/ml), and it was chosen to continue the research (Table 2). Genetic testing revealed that E coli NRC1169 is the powerful strain No. 4.

Table 1. Qqualitative estimation of alpha amylase isolated strain

Isolate no	Amylase test
1	+
2	-
3	+
4	++
5	+
6	+
7	-
8	+
9	+
10	-
11	-
12	+

Table 2. Qquantitative estimation of amylase activity of isolated strain

Isolate no	Amylase activity (u/ml)
1	123.21
3	67.89
4	189.51
5	109.19
6	89.12
8	95.34
9	110.43
12	93.48

Effects of temperature and pH on amylase activity

The results showed that Amylase activity increased as pH increased, peaking at 0.297 U/ml at pH 7, then declining at higher pH values to 0.116, 0.059 U/ml, and pH 8 and 9 respectively (Figures 2 and 3). The amino acid and carboxylic acid components of the enzyme changed in ionic nature as a result of the reaction mixture's altered pH. It changed activity via affecting the conformational state of the enzyme and the catalytic site. Additionally, the temperature had an impact on the production process, with the maximal enzyme yield (1.51 U/ml) occurring at 40 ℃ and gradually declining until 50 ℃.

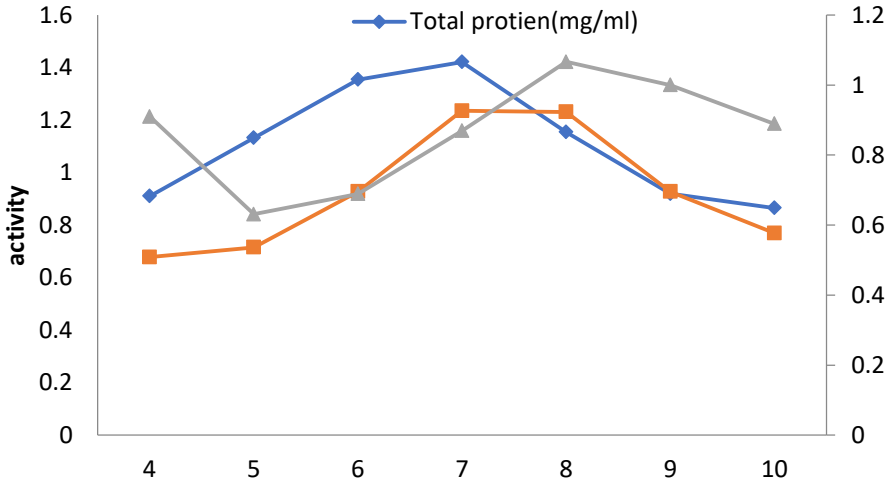

Figure 2. Effect of different pH on amylase enzyme production by *E. coli*

Fractionation of sulphate of ammonium

According to the previously described enzyme assay method, the ammonium sulphate was added at various saturation ratios of 20, 40, 60, and 80% to achieve the optimal ratio of ammonium enzyme activity. There was evidence of protein concentration (mg/ml) absorption at 595 nm. To

each 20 ml of the crude amylase, ammonium sulphate was gradually added, along with salt, using a magnetic stirrer in an ice bath. The mixture was then centrifuged for 25 minutes at 6000 rpm. The precipitate was dissolved in 5 cc of distilled.

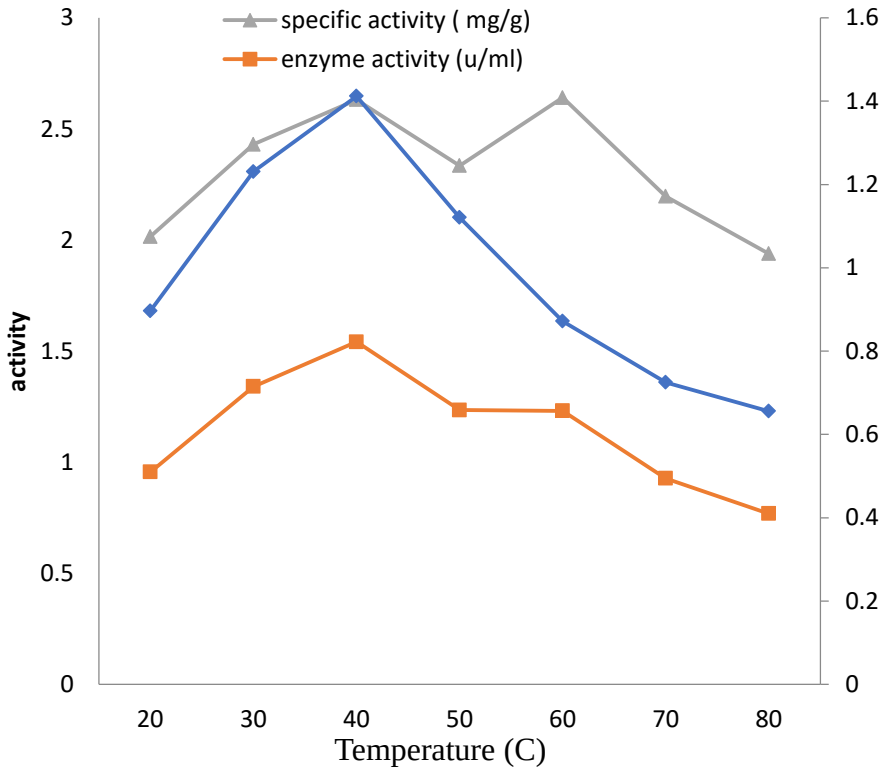

Figure 3. Effect of temperature on α amylase enzyme production obtained from E. coli

Immobilization of enzymes

The following procedures were used to immobilize the amylase enzyme. The enzyme was dissolved in 1 mL of 0.1 M Tris-HCl buffer, pH 9.5, before being added to 5.0 mg of 100 nm chitosan nanoparticles. In the end, 25 L of EDC with a 100 mg/mL concentration was added to the solution. For 48 hours at 4 ℃, the mixture was slowly mixed. To remove the immobilized enzyme, the solids were separated and rinsed with the same buffer.

Amount of dye's impact

Measurements were made on Madder dye's comparative extractability at concentrations ranging from 20 to 80 g/L using microwave

heating for 5 minutes and conventional heating for an hour at boiling temperature. It demonstrated that whether utilizing microwave heating or traditional heating, the color strength (K/S) of the dyed wool fibers rose as the concentration of dye increased (Figure 2). It demonstrated that 40 g/l was the optimal concentration for extraction by microwave heating.

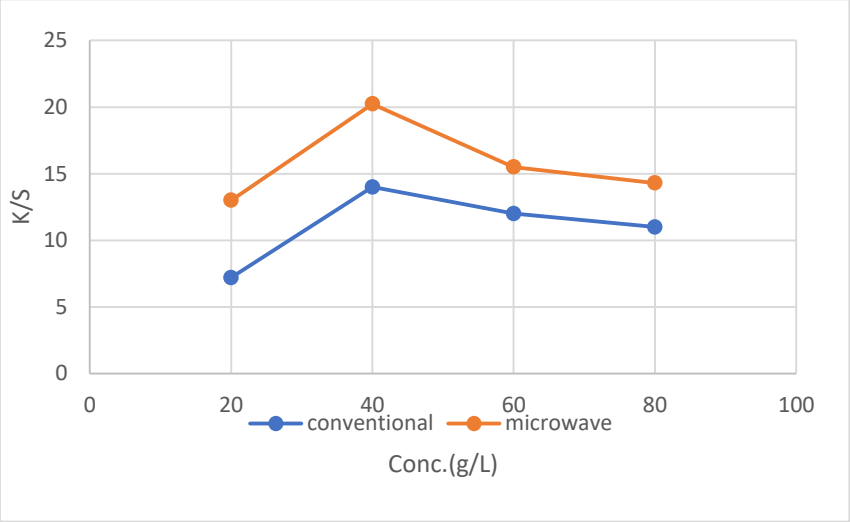

Figure 4. Effect of dye amount

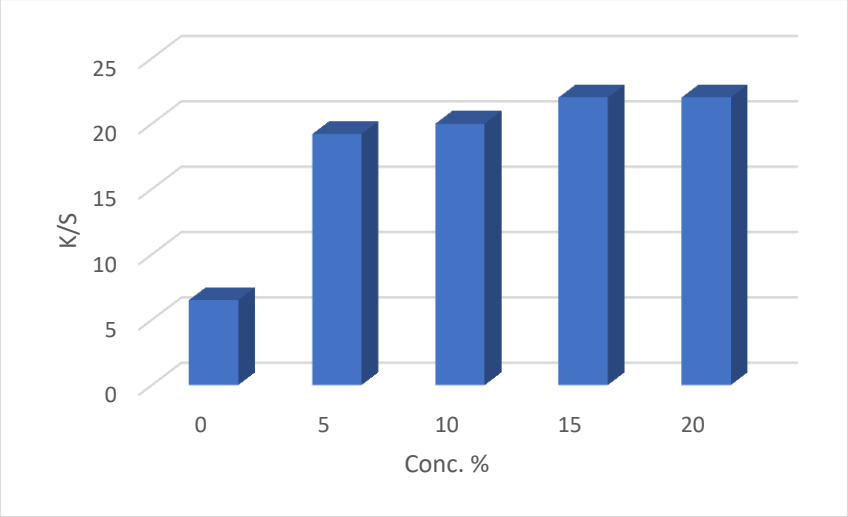

Figure 5. Effect of enzyme on the colour strength (K/S) for wool fibres dyed with madder natural dye

Dye concentration in dyeing procedures

Natural dyes derived from madder at various strengths (20-80 g/l) at a liquid ratio of 1:100 are used to color wool fibers. Wool fibers were

colored by five minutes of microwave heating. The findings indicated that increasing the dye concentration to 60g/l and drying by microwave technique enhanced the K/S.

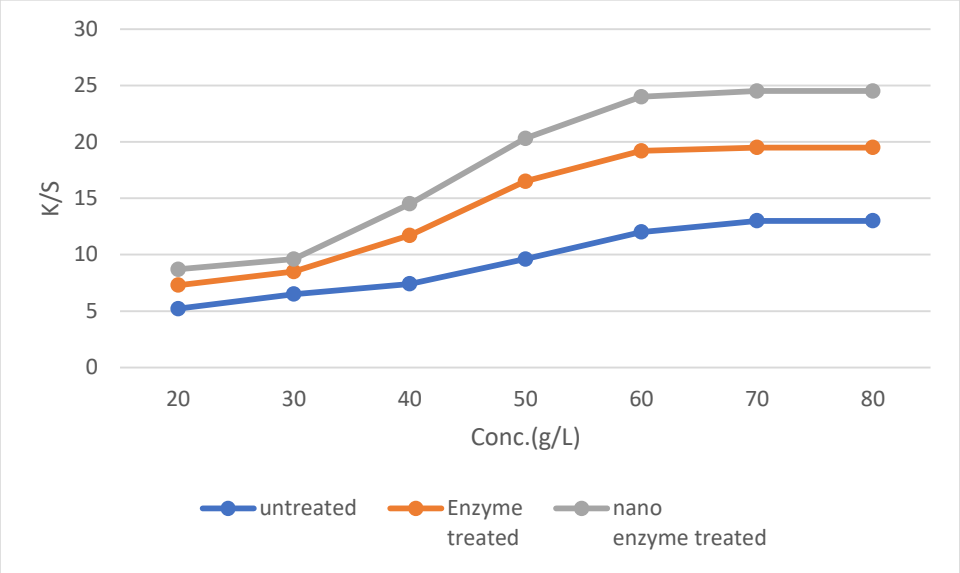

Figure 6. Effect of concentration of dye in dyeing processes

Dye-infused fibers showed noticeably better levelness. When using conventional heating, the absorbed dye is irregularly distributed on each fiber and is focused mostly in the outer portions. A higher level of dye penetration resulted with the use of microwave heating. The concentration of the dyestuff on the surface of the fibers increased and became more homogeneous as a result of microwave heating, allowing it to diffuse into the interior of the fibers.

pH of the dye bath

When expressed as K/S, the results showed that the pH values of 3 to 7 in the dye bath had a significant impact on the wool fibers' ability to take color (Figure 4). The findings showed that at pH 5, microwave irradiation improved dye ability. The waves connecting dye molecules and wool fibers were thought to be responsible for the dye bath's effect on pH level.

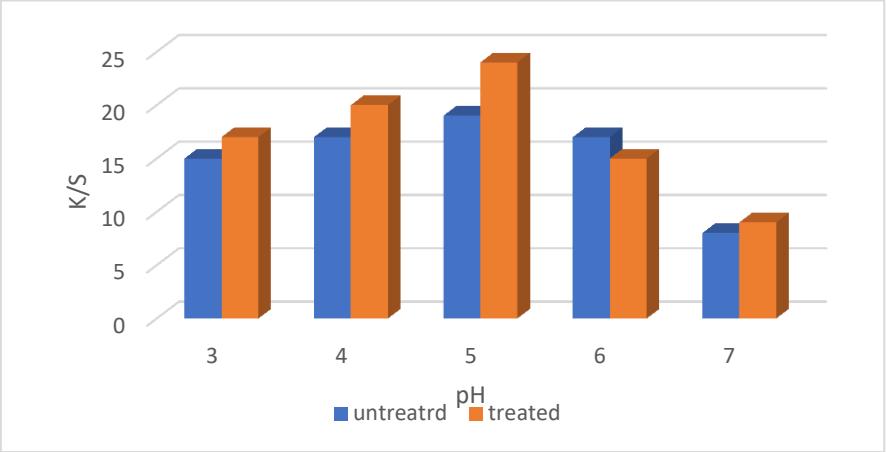

Figure 7. Effect of pH of the dye bath on K/S of wool fibers dyed by microwave

Effect of microwave pretreatment on dyeing

The fiber's coloring was improved by the enzyme treatments. It either formed chemical connections with the polypeptide chain's terminal -NH2 or -COOH groups or with the functional groups found in the side chains of the constituent amino acids. The treated samples showed higher K/S values than the untreated samples, and the enzyme pretreatment improved the treated fibers' dyeing abilities in comparison to untreated colored wool fiber dyes.

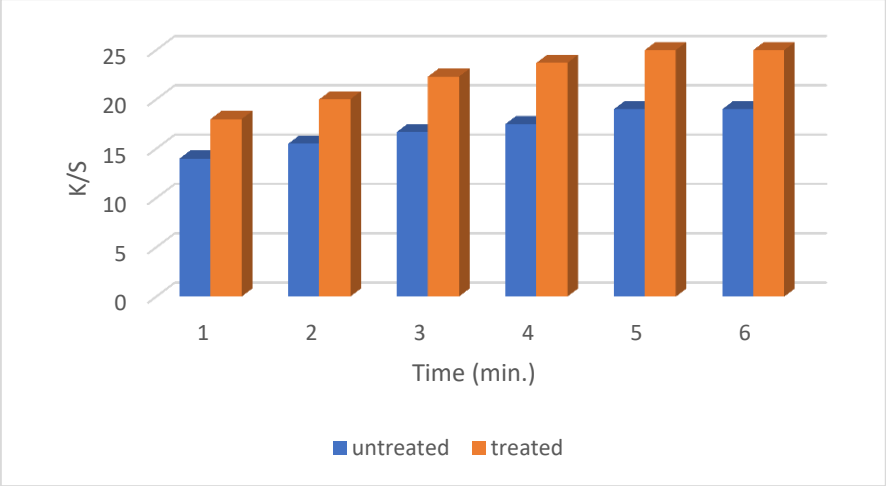

Figure 8. Effect of time on dyeing Wool Fibres by microwave method on color strength (K/S)

Effect of enzyme concentrations on madder-dyed wool fibers

The results showed that at 15% concentration of wool fibers dyed with madder by microwave, pretreatment using (5-20%) convergence of enzyme generated the most notable estimation of color strength (K/S). The outcomes demonstrated the impact of time dyeing wool fibers that were microwave-pretreated and madder-dye. The findings indicated that the color strength reached its greatest values at five minutes. It demonstrated the impact of the dyeing pH in a bath of wool fibers that had been microwave-treated and dyed (Figure 4).

Measuring and testing antibacterial activity

The nutrient agar medium (g/L) contained 20 g of agar at pH 7.5 that was prepared and autoclaved at 121 ℃ for 20 min. It also contained peptone, beef extract, yeast extract, and 1.5 g of beef extract. Equal layers of nutritional agar were made on sterilized petri dishes. Gram positive and gram negative bacteria, as well as the fungus *Candida albicans* and *Aspergillus niger* were used in the test organisms. They were cultured overnight at 37 ℃ in 2 mL of nutrient broth. Under sterile conditions, wool samples were placed on top of the seeded medium. The zones of inhibition were evaluated following an overnight incubation at 37 ℃.

Table 3. Antimicrobial activity for dyed wool fibers at different concentrations of enzyme

	Inhibition Zone			
Sample no.	*E. coli*	*B. subtilus*	*C. albicans*	*S. aureus*
Control				1
1 (5%)	1.5	1.2	0.9	1.1
2 (10%)	2.7	2.3	1.1	0.9
3 (15%)	1.3	1.1	0.8	1
4 (20%)	1.2	1.1	0.7	1.1

Fastness characteristics results

On wool fibers, the investigational dyes' fastness characteristics and color yield were assessed. Because the dye was fixed as a result of the treatment, color fastness to rubbing, washing, and perspiration of all dyes was found to be excellent to good and roughly the same in the microwave and conventional procedures (Table 4). Additionally, it was discovered that the light fastness for all dyed fibers was almost equivalent for the two procedures, but that treated samples had faster light fastness overall than untreated samples.

Table 4. Fastness properties of dyed wool pretreated with enzyme and dyed with madder natural dye

Dyed samples	Fastness to rubbing		Wash fastness			Fastness to Perspiration						Light
						Alkaline			Acidic			
	Dry	Wet	Alt	SC	SW	Alt	SC	SW	Alt	SC	SW	
Treated	5	5	4-5	4-5	4-5	5	4-5	4-5	5	5	5	7
Untreated	3	3	4	3	3	4	3	3	4	4	5	6

Alt = change in color, SC = staining on cotton, SW = staining on wool

Discussion

The collision of dye molecules with fiber particles depends on how quickly the particles move through the dye solution (Zhan and Zhao, 2009). In microwave dyeing, the dielectric and warming properties of materials are taken into account. The term "dielectric property" refers to inherent electrical characteristics that affect the dipoles' microwave field and coloration as a result of the color's dipolar pivot. In the high repetition microwave field, the fluid dye arrangement contains two polar segments. It affects the dye molecules and water molecule's colorful vitality (Xue and Jin-xin, 2011).

Crude enzyme was pre-incubated for 30 min at various temperatures (20-80C) prior to enzyme measurement, quickly cooled on ice, and residual activity was assessed under normal assay conditions. This was done to quantify thermostability. The half-life of -amylase was calculated by incubating the raw enzyme at 60 ℃ and measuring the residual activity under the parameters of the standard assay (George and George, 2020).

After incubating at 27 and 40 ℃ for 6 hours, the residual amylase activity was still maintained at over 78% of activity, however amylase only displayed about 50% of iactivity when incubated at 50, 55, and 60 ℃ for the same amount of time. At all temperatures that were examined, the amylase still had more than 50% of its initial activity after 24 hours. Only 18% of the enzyme's initial activity was still present at 70 ℃. Below 50 ℃, the enzyme exhibited good stability. These findings suggested that the amylase might be employed in biotechnological applications at any temperature between 27 and 60 ℃. By incubating the enzyme with various pH buffers for 30 min at 60 ℃ prior to the enzyme test, the effect of different pH 6 on enzyme stability was examined. The residual activity was then measured under the usual assay conditions. By assessing the residual activity under typical assay conditions at 60 ℃, the effect of pH on enzyme for thermal-stability was discovered.

Amylases often remain stable over a broad pH range of 4.0 to 10. However, -amylases with stability in a constrained range have also been documented (Gupta *et al*., 2003), with bacterial amylase remaining active and stable for 24 hours at a temperature of 40 ℃ between pH 4 and 8.5. These findings revealed that the enzyme was not very pH-sensitive. The

enzyme may therefore find extensive use in a variety of industrial fields.n The enzyme serves as a catalyst at the end of the process, remaining intact, and speeds up the reaction by lowering the activation energy (Mojsov, 2011).

Two isolates were chosen for physiological and biochemical responses because they produced the most amylase. An appropriate substrate for fermentation revealed the correct generating isolates. The crude enzyme's partial purification, characterization, and use of nano-chitosan as a carrier were noted. The pre-treatment of wool fibers dyed with madder natural dye was researched in the field of textiles using a partially purified enzyme. The fermentation medium with the highest level of activity contained starch, 10 peptone, 10 yeast extract, 5 ammonium sulfate, 0.25 magnesium. The results showed that the enzyme-free and immobilized wool fiber samples had better color strength and fastness qualities than the untreated fibers. The amylase-treated fibers' antibacterial activity showed greater suppression than the untreated fibers.

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