3.0 MATERIALS AND METHODS

This section describes the materials and methods used in the present study.

3.1 MATERIALS

Different agro-industrial waste materials (green gram, rice bran, and wheat bran) were collected from the local market and processed using USA standard sieve set of Nos. 7, 10, 14, 18 and 50 to obtain mean particle size of 2.8–2.0; 2.0–1.4; 1.4– 1.0 and 1.0–0.3 mm and stored till further use. Sephadex G-100 and gel filtration matrix having medium viscosity of 500 m Pas, sodium alginate were purchased from Sigma (USA). All other chemicals used were of analytical grade procured from Qualigens, India.

3.1.1 SAMPLE COLLECTION

For isolation of amylase enzyme from Aspergillus species, samples were collected from various places located around Hyderabad, and dump yards of Gayatri Starchkem, Hyderabad, Andhra Pradesh, India and brought to laboratory and these samples were stored at 4°C till further use.

3.1.2 ENRICHMENT OF SOIL MICROBIAL ORGANISMS

One gram of soil sample was suspended in 9.0 ml of sterile distilled water and mixed well for one hour at room temperature and 1.0 ml of suspension was used to inoculate in 50 ml of Potato Dextrose Broth (PDB) medium consisting (g/l) of Potato infusion 4 g/l and Dextrose 20 g/l. The pH of the medium was adjusted to 5.5 using 0.1N Hydrochloric acid or 0.1N Sodium hydroxide solution. The culture was incubated at 30°C and at 200 rpm. After 24 h of incubation, the resultant culture was serially diluted using sterile distilled water and 0.1 ml of this culture was spread on PDA starch plates containing starch (10g/l).This culture was incubated at 30°C for 48 h and observed for growth. Each developed colony was picked up and maintained on starch containing PDA medium till further use.
3.2 SCREENING FOR AMYLOLYTIC ACTIVITY

Amylolytic activity of isolated strains was screened by plating them on defined medium consisting (g/l) of Sucrose 30.0, Starch 10.0, Magnesium sulphate 0.5, Potassium chloride 0.5, Potassium phosphate 1.0, Ferrous sulphate, 0.01 and Peptone 5.0. These plates were incubated at 30 °C for 24 h. Fungi showing clear zones of hydrolysis on PDA starch agar plates after pouring Iodine solution were identified as amylase producers. Based on hydrolysis zone, few colonies were selected and maintained on the PDA medium slants at 4 °C. One of the colonies which were showing more amylolytic activity was designated as MK 07 and was selected for further studies.

3.2.1 CHARACTERIZATION OF AMYLASE PRODUCING ISOLATE

The cultural, morphological and physiological characteristics of the strain MK 07 were investigated by using various media and biochemical reactions according to Bergey’s Manual of Determinative Bacteriology.

3.2.2 EFFECT OF SODIUM CHLORIDE ON GROWTH

To study the effect of sodium chloride on growth of Aspergillus strain, the isolate was cultured in a basal medium (BM) consisting (g/l) of Magnesium sulphate – 0.2, Ammonium di hydrogen phosphate – 1.0, Potassium di hydrogen phosphate – 1.0 in different flasks supplemented with varying concentrations of sodium chloride (%) 2.0 5.0, 7.0, 8.0 and 10 % respectively. Growth was observed after incubating these flasks at 30°C for 48 h and later on cell optical density was measured in an UV Spectrophotometer at 600 nm to monitor the effect of sodium chloride on growth of the isolated strain against a medium without culture as a blank.
3.3 **BIOCHEMICAL CHARACTERIZATION**

The following biochemical tests were performed to characterize the selected organism.

**3.3.1 CITRATE UTILIZATION TEST**

Citrate utilization test was performed to determine the ability of the isolate to utilize citrate as sole carbon source using Simmon’s citrate agar medium. In this medium sodium citrate was used as carbon source and ammonium ions as nitrogen source along with bromothymol blue as indicator. Citrate utilization results were analyzed by observing the change of colour of the medium from green to blue against control.

**3.3.2 UREASE TEST**

Urea utilization ability of isolated MK 07 strain was analyzed by growing the isolated strain in 1% urea supplemented YPD medium. The resulting rise in the pH of the medium due to the hydrolysis of urea by urease and subsequent release of ammonia was detected by the change in color of the indicator phenol red to dark pink.

**3.3.3 HYDROGEN SULFIDE (H₂S) PRODUCTION**

H₂S production is a natural by-product of metabolism in some microorganisms. The production of H₂S was detected by growing isolate in SIM (Sulfide, Indole and Motility) medium and subsequent appearance of ferrous sulfate black precipitate against control experiment.

**3.3.4 NITRATE REDUCTION TEST**

The nitrate reduction ability of the isolate was measured by adding the sulfanilic acid (Solution A) and α-naphthylamine (Solution B) to the incubated culture solution and subsequent red colour development against the control (medium without inoculum).
3.3.5 **PHENYLALANINE TEST**

This test differentiates microorganisms that possess the enzyme phenylalanine deaminase. The medium contained the amino acid phenylalanine. The enzyme phenylalanine deaminase converted phenylalanine to phenylpyruvic acid by the removal of the terminal amide group of the amino acid. This phenyl pyruvic acid was detected by the addition of 10 % ferric chloride solution. Change in color from yellow to green indicated positive result. Organism was inoculated and, an uninoculated tube was kept as a control. After incubation, a few drops of 10 % ferric chloride solution were poured over the growth and the color change was observed.

3.3.6 **STARCH HYDROLYSIS**

Starch hydrolysis character of the isolated strain, MK 07 was determined by growing the microbial strain on starch-agar based plates and subsequent identification by starch-iodine test. 1.0 % starch-agar plates were prepared under sterilized conditions and these plates were inoculated by spreading 1 ml of culture with a sterilized glass spreader of the isolated strain. These spread plates were incubated for 48 h for development of colonies. These plates were then subjected to iodine test by pouring the iodine solution on the plates. Starch hydrolysis by colonies was detected by the presence of clear zones against a blue background.

3.4 **ANALYTICAL METHODS**

3.4.1 **MEASUREMENT OF AMYLASE ACTIVITY**

The activity of the alpha amylase was determined by the Bernfeld (1955) procedure using soluble starch (Sigma chemical, USA) as a substrate. The reaction mixture containing 1 ml of 1% substrate (w/v) in 0.02 M Phosphate buffer (pH 6.8), 0.5 ml of crude enzyme and 0.5 ml of the buffer, was incubated for 5 mins at 55°C. The reaction was stopped by adding 2 ml of a solution 3, 5-di nitro salicylic acid
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(DNS), followed by cooling to room temperature. The concentration of the reducing sugar was measured at 540 nm in an UV- Vis Spectrophotometer using maltose as standard. The blank contained 0.5 ml, 0.02M phosphate buffer (pH 6.8), 0.5 ml crude enzyme and 1 ml of 1% starch solution. One unit (U) of alpha amylase is defined as the amount of enzyme that releases 1 micro mole of reducing sugar as maltose per minute under the assay condition and is expressed as U/g and U/ml of substrate in solid state and submerged state respectively. (Adinarayana et al, 2005).

3.4.2 CONSTRUCTION OF STANDARD GRAPH FOR MALTOSE

3.4.2.1. STOCK SOLUTION

Maltose was used as standard reference for amylase activity. One mg/ml maltose solution was prepared and used as stock solution. 10 appropriate dilutions from 0.1-1.0 were made from standard stock solution. 1 ml of each dilution and 1 ml of DNS solution was added in each test tube and blank was made with 1 ml of distilled water and 1 ml of DNS solution. These tubes were placed in boiling water bath for 5 mins and cooled to room temperature. The contents of the test tubes were diluted upto 10 ml with distilled water. All the tubes were mixed well and optical density of the solution was measured at 540 nm. A standard curve was constructed taking concentration of maltose (mg/ml) on X-axis and corresponding optical density on Y-axis (Lonsane et al, 1990).

3.4.3 PROTEIN ESTIMATION

The protein content of the enzyme preparations was estimated by Lowry method using Bovine serum albumin as standard. 1 mg /ml stock solution is prepared and from that stock solution various dilutions ranging from 0.1 mg/ml -1.0 mg/ml were prepared and
standard plot was performed. From each dilution 0.2 ml of solution was taken in different test tubes and to each tube added 2 ml of alkaline copper sulphate reagent and this mixture was mixed well and incubated at room temperature for 10 mins. Then 0.2 ml of Folin Ciocalteau reagent was added to each tube and incubated for 30 mins. After incubation absorbance was read at 595 nm in an UV Vis Spectrophotometer. Then a standard graph was plotted with concentration on X-axis and optical density on Y-axis (Adinarayana et al, 2005, Bernfeld, 1955).

3.4.4 **GLUCOSE ESTIMATION**

Glucose content in fermentation broth was estimated using DNS method as reported by Prakasham et al., (2007). In short, 0.1 ml of the cell free broth was diluted to 1.0 ml with distilled water and to this 2.0 ml of 3–5 dinitro salicylic acid was added and mixed thoroughly. The contents were then boiled for 5 min and diluted the volume to 10.0 ml with distilled water. The final solution absorbance was read at 540 nm using UV–Visible spectrophotometer (Shimadzu) and the concentration of glucose was calculated using glucose standard curve (Prakasham et al, 2005).

3.5. **ENZYME EXTRACTION**

In case of submerged fermentation, the fermented medium was centrifuged at 5000 rpm for 15 min and the supernatant was separated used as the crude enzyme solution while, in case of solid state fermentation, the enzyme was extracted according to the method described by Nagamine et al., (2003). Fermented medium was mixed thoroughly with 50 mM glycine–Sodium hydroxide buffer, pH 11 for 30 min and the extract was separated by squeezing through a cloth. This process was repeated three times and the extracts were pooled together and then centrifuged (10,000x g, 10 mins, 4°C) and the clear supernatant was used as enzyme source for amylase assay (Lonsane et al, 1990).
3.6 ENUMERATION OF FUNGAL SPECIES MK 07 SPORES

To estimate the viable spores count, the encapsulated fungal spores were released from the beads by dissolving 5 beads in phosphate buffer (pH 7.0) for 30 min followed by gentle homogenization. The fungal spore count in this solution was measured after serial dilution and by on plating agar based PDA medium using standard plate count method after incubating for 24 h.

3.7 MEDIUM FOR AMYLASE PRODUCTION

In case of submerged fermentations, the defined medium and growth conditions mentioned elsewhere in materials and methods was used for amylase production studies. Whereas, for solid state fermentation, different agro material mentioned in section 3.1 were used as substrate unless otherwise mentioned.

3.8 PREPARATION OF SOLID MEDIUM FOR AMYLASE PRODUCTION.

Ten grams of selected substrate was taken in 250 ml Erlenmeyer flasks and to this a predetermined quantity of water was added, mixed thoroughly and autoclaved at 121 °C for 15 min at 15 Lbs. After cooling the flasks to room temperature, the flasks were inoculated with a loopful of fungal spores of 24 h grown culture broth under sterile conditions. Contents of the flasks were well mixed and incubated at predetermined temperatures. After 48 h of incubation enzyme was extracted using glycine sodium hydroxide buffer and used for further studies.

3.9 OPTIMIZATION OF PROCESS PARAMETERS

The process parameters such as pH, incubation temperature, inoculum concentration and RPM were screened and selected based on classical approach using defined medium under standard experimental conditions mentioned elsewhere in this section.
3.9.1. **SCREENING OF CARBON AND NITROGEN SOURCES FOR AMYLASE PRODUCTION.**

For selection of the best medium components and their concentrations, different carbon sources (glucose, ribose, starch, xylose, maltose, cellulose, mannitol, sucrose, arabinose, fructose, mannose) and nitrogen sources such as (potassium nitrate, ammonium sulphate, ammonium chloride, ammonium nitrate, yeast extract, beef extract, corn steep liquor, casein, soya bean meal, ammonium persulphate, peptone, sodium nitrate, urea, tryptone) sources were selected based on Plackett-Burman approach. The selected carbon and nitrogen sources were replaced with glucose, yeast extract and peptone in growth medium. All these experiments were carried in Erlenmeyer flasks of 250 ml containing 100 ml of medium under standard fermentation conditions, unless otherwise stated (Pandey et al, 1990; Ellaiah et al, 2002, Prakasham et al, 2005).

3.10 **OPTIMIZATION OF SOLID STATE FERMENTATION**

Ten grams of wheat bran was transferred to each 250ml cotton plugged Erlenmeyer flask. The wheat bran was moistened with phosphate buffer (0.02M) in the ratio of 1:1. The flasks were sterilized in the autoclave and then cooled at room temperature. One ml of the fungal inoculum was aseptically transferred to each flask. The flasks were placed in the incubator at 30°C for 48 hrs. All the experiments were run in parallel in triplicates (Ellaiah et al, 2002).

3.10.1 **EXTRACTION OF THE ENZYME**

After the incubation period, 100 ml of phosphate buffer (0.02M) along with 0.02% Tween 80 was transferred to each flask containing fermented bran. The flasks were rotated in the incubator
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shaker at 200 RPM for one hour. After one hour, the contents of the flask were centrifuged in the centrifuge machine at 5000 RPM for 15 minutes. The substrate free suspension was used for the estimation of biomass and alpha amylase (Lonsane et al, 1990).

3.10.2. OPTIMIZATION OF VARIOUS PARAMETERS FOR ALPHA AMYLASE PRODUCTION BY SOLID STATE FERMENTATION

3.10.2.1. SELECTION OF SUBSTRATE
Different agricultural biproducts such as wheat bran, rice bran and green gram husk were tested for the production of alpha amylase by isolated Aspergillus niger strain MK 07.

3.10.2.2. EFFECT OF TEMPERATURE
The effect of varying ranges of temperature on the production of alpha amylase by isolated Aspergillus niger strain MK 07 was investigated. The fermentation was carried out at 25, 30, 35, 40, 45 and 50°C respectively. Biomass formation and alpha amylase production was studied.

3.10.2.3 EFFECT OF INITIAL pH
Effect of varying pH ranges of 4.5, 5.0, 5.5, 6.0 and 6.5 on the growth of isolated Aspergillus niger strain MK 07 and enzyme production was studied.

3.10.2.4 EFFECT OF INCUBATION PERIOD
Growth of isolated Aspergillus niger strain MK 07 and production of amylase were carried out for 120 hrs at 30°C . The samples were collected every 12 hours and observed for the production of biomass and amylase enzyme.
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3.10.2.5 **EFFECT OF DIFFERENT CARBON SOURCES**

The effect of different carbon sources on the growth of fungal strain and production of alpha amylase was studied. Different carbon sources such as starch, glucose, sucrose, maltose, xylose and lactose were studied.

3.10.2.6 **EFFECT OF DIFFERENT NITROGEN SOURCES**

The effect of different nitrogen sources on the growth of isolated *Aspergillus niger* strain MK 07 and production of alpha amylase was studied. Inorganic nitrogen sources like ammonium sulphate, ammonium nitrate, ammonium chloride, sodium nitrate were studied.

3.10.2.7 **EFFECT OF DIFFERENT INOCULUM SIZES**

The effect of different inoculum sizes were investigated for the production of alpha amylase by the isolated *Aspergillus niger* strain MK 07.

3.11 **SUBMERGED FERMENTATION**

Submerged fermentation experiments were carried out in shake flask and Fermentor. Individual experiments carried out are mentioned below.

3.11.1 **EFFECT OF CORN STEEP LIQUOR ON SYNTHETIC MEDIA**

The effect of various concentrations of Corn Steep Liquor (CSL) on the synthetic media was studied to analyze the effect of additional supplementation of CSL on amylase activity and biomass by the isolated *Aspergillus niger* strain MK 07.

3.11.2 **EFFECT OF TEMPERATURE**

The effect of temperature on the production of alpha amylase by isolated *Aspergillus niger* strain MK 07 was investigated. The fermentation was carried out at varying temperatures like 25,
30, 35, 40, 45 and 50°C respectively. Biomass formation and alpha amylase production was studied.

3.11.3 **EFFECT OF INITIAL pH**

Effect of initial pH 4.5, 5.0, 5.5, 6.0 and 6.5 on the growth of isolated *Aspergillus niger* strain MK 07 and enzyme production was studied.

3.11.4 **EFFECT OF INCUBATION PERIOD**

Growth of isolated *Aspergillus niger* strain MK 07 and production of amylase were studied for 120 hrs at 30°C. The samples were collected every 12 hours and observed for the production of amylase.

3.11.5 **EFFECT OF DIFFERENT CARBON SOURCES**

The effect of different carbon sources on the growth of isolated *Aspergillus niger* strain MK 07 and production of alpha amylase was studied. Different carbon sources such as starch, glucose, sucrose, maltose, xylose and lactose were studied.

3.11.6 **EFFECT OF DIFFERENT NITROGEN SOURCES**

The effect of different nitrogen sources on the growth of isolated *Aspergillus niger* strain MK 07 and production of alpha amylase was studied. Inorganic nitrogen sources like ammonium sulphate, ammonium nitrate, ammonium chloride, sodium nitrate were studied.

3.11.7 **EFFECT OF DIFFERENT INOCULUM SIZES**

The effect of different inoculum sizes were investigated for the production of alpha amylase by the isolated *Aspergillus niger* strain MK 07.
3.12 REACTOR STUDIES

The isolated *Aspergillus niger* strain, MK 07 was grown in 2 L B-Braun Fermentor (Germany) containing one liter of medium consisting of synthetic media and glucose as a carbon source in varying ranges from 10 to 40 g l⁻¹ at pH 5.0 by incubating at 35.0°C and at 150 rpm for 12 h. Growth was measured by estimating the absorbance at 600 nm. The dry weight of cell mass was estimated from a previous calibrated curve of absorbance vs cell mass according to (Rani *et al.*, 2003). The cell free supernatant was used to measure glucose concentration and amylase activity. All assays were carried out in triplicate.

3.13 ENZYME PURIFICATION

All enzyme purification steps were carried out at 0 to 4 °C.

3.13.1 AMMONIUM SULPHATE PRECIPITATION

The crude broth obtained after fermentation was centrifuged at 5000 X g for 15 min to remove the cell biomass. Solid ammonium sulphate was added slowly to the culture supernatant to get 60 % saturation, stirred for 60 min and left for overnight at 4°C. The precipitate was harvested by centrifugation at 10,000 X g for 10 min, dissolved in 50 mM glycine-sodium hydroxide buffer and dialyzed against same buffer overnight (4°C). The dialyzed sample was then assayed for amylase activity and glucose content (Lonsane *et al*, 1990).

3.13.2 SEPHADEX G-100 CHROMATOGRAPHY

Dialyzed enzyme was loaded on to a column of sephadex G-100 (1.5 x 90 cm) previously equilibrated with 50 mM glycine-sodium hydroxide buffer (pH 11) and then eluted at a flow rate of 10 ml/h with the same buffer containing sodium chloride gradient from 0.1 to 1 M and collected 1 ml volume fractions. The absorbance of fractions was checked at 600nm. Those fractions which showed absorbance were assayed for amylase activity with starch as substrate. Amylase
active fractions were pooled and concentrated for further characterization (Sivaramakrishnan et al, 2005).

3.13.3 POLYACRYLAMIDE GEL ELECTROPHORESIS

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Prakasham et al, (2005) using a 10 % crosslinked polyacrylamide gel on a Tarson gel electrophoresis unit (Tarson, India). Electrophoresis was carried out at constant voltage (300V and 60 mA) for 90 min at room temperature. Silver staining was performed to visualize protein bands on the gel. Native PAGE was performed according to the method of Prakasham et al, (2005) with Tris/Glycine buffer, pH 8.3. Coomassie brilliant blue (0.1 %) staining was used to detect the presence of protein bands if any on the gel.

3.13.4 SILVER STAINING

Gel was soaked for 10 min in fixing solution containing 40 ml methanol, 13.5 ml formalin, 46.5 ml water. Then the gel was washed twice in deionised water and soaked for 1.0 min in 0.02 % Na₂S₂O₃ (Sodium thiosulfate) solution. Subsequently the gel was washed twice in water for 20 sec and again soaked in 0.1 % AgNO₃ (Silver nitrate). After 10 min, the gel was removed and rinsed with water followed by developing solution (3.00 % sodium carbonate, 0.05 % formalin, 0.000016 % Na₂S₂O₃) and soaked in fresh developing solution until band intensities were adequately visible (~1-3 min). 2.3 M citric acid (or 6 g solid citric acid) solution was added to stop the reaction. The gel was finally washed in water, and then soaked in water for 30 min before drying (Adinarayana et al, 2005; Prakasham et al, 2005).
3.14 ENZYME CHARACTERIZATION

3.14.1. DETERMINATION OF THE pH OPTIMUM AND pH STABILITY

The pH optimum for purified amylase was assayed by analyzing its activity in the pH range of 5 to 12 using starch as a substrate and buffer systems of 0.05 mol l\(^{-1}\) phosphate buffer for pH 5.0 to 7.5, Tris-HCl for pH 8.0 to 9.0, glycine-Sodium hydroxide for pH 9.5 to 11.0, sodium phosphate for 11.5 to 12.0 and sodium carbonate for 12.5 to 13.0. pH stability study of the protein was analyzed by pre-incubating 5 ml of purified enzyme in 3.5 ml of selected pH buffer at 37 °C for 1 to 48 h and subsequent analysis of residual activities under standard assay conditions.

3.14.2 DETERMINATION OF OPTIMUM TEMPERATURE AND THERMAL STABILITY

To study the temperature optima of enzyme, the enzyme reaction mixture was incubated at different temperatures ranging from 35 °C to 75 °C in glycine-sodium hydroxide buffer (pH 11.0) and measured amylase activity using starch (1 %) solution as substrate. For determining thermal stability, the enzyme was pre-incubated for 1.0 h at different temperatures ranging from 35 to 90 °C and the relative activity was measured under standard assay conditions after incubating with starch as substrate (Haq et al, 1997).

3.14.3 THERMO-INACTIVATION STUDIES

Thermo inactivation assays were carried out by pre heating 950 µl of standard buffer at the corresponding temperature, then adding 1 µg protein in 50 µl of the same buffer and pre-incubating the mixture at the same temperature. Samples were collected every 1.0 h at 75, 80, 85 and 90°C and cooled to 70°C before analyzing the amylase activity.
3.15 **EFFECT OF VARIOUS METAL IONS**

Various metal ions like Ca$^{2+}$, Co$^{2+}$, Cu$^{2+}$, Mg$^{2+}$, Mn$^{2+}$, Fe$^{2+}$, Hg$^{2+}$, Na$^{+}$ and Zn$^{2+}$, impact on the enzyme catalytic behaviour was studied by pre-incubating purified enzyme in a specified ion (10mM final concentration) containing buffer solution. After 1 h of incubation, starch was added and relative activity of the enzyme was measured as described above.

3.16 **EFFECT OF SURFACTANTS AND OXIDATIVES ON AMYLASE ACTIVITY**

The impact of 1.0% final concentration of different surfactants and oxidatives (SDS in w.v$^{-1}$, Tween-80, Triton X-100 in v.v$^{-1}$ and Hydrogen peroxide in v.v$^{-1}$) on amylolytic activity of the purified alpha amylase was studied by pre-incubating the enzyme in above surfactant solutions at room temperature before being tested for amylase activity. After 4 h incubation, starch was added and relative activity of the enzyme was measured as described above. A parallel control was kept with enzyme and buffer with substrate and the control activity value was considered as 100%.
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3.17. **AMYLASE APPLICATION POTENTIAL EVALUATION**

3.17.1 **ALPHA AMYLASE AS DESIZER**

A clean and stiffed cloth with starch over that was used for the present study. Equal sizes of pieces were used for the study and they were weighed on an electric balance (5x5 inch). The cloth strip was then dipped in 100 ml of enzyme solution (pH 6.5) and then placed in the incubator at 70-80°C for 1.0 hr. After the time interval, the cloth strip was washed with tap water and then oven dried. After drying the cloth strip was again weighed.

Weight of starch removed = Initial weight - final weight

The % removal of starch was calculated by applying the following formula.

\[
\text{% of Desizing} = \frac{\text{Wt of starch removed by enzyme}}{\text{Total starch present on the cotton strip.}}
\]

Total starch was calculated by hydrolyzing the starch with 0.1N sulphuric acid.

The following parameters were studied for desizing of the cotton cloth.

3.17.1.1. **EFFECT OF ENZYME CONCENTRATION:**

The effect of enzyme concentration on the desizing of the cotton cloth by crude and partially purified enzyme was investigated. The concentration of the enzyme was varied from 100-500 U/ml/ min.

3.17.1.2. **EFFECT OF TEMPERATURE:**

The effect of temperature (25- 50°C) on the desizing of cotton cloth by crude and partially purified enzyme was studied.
3.17.1.3 **EFFECT OF pH:**

The effect of different pH (4.0-9.0) on the desizing of cotton cloth by crude and partially purified enzyme was studied.

3.17.1.4. **TIME PROFILE FOR THE DESIZING OF THE COTTON CLOTH**

The rate of desizing of cotton cloth at various time intervals by crude and partially purified alpha amylase was carried out. The cloth was treated with crude and partially purified enzymes at 80°C for 75 mins.