CHAPTER 7

APPLICATION OF ALPHA AMYLASE PRODUCED BY
BACILLUS LICHENIFORMIS SPT -27
7.1. INTRODUCTION:-

Alpha amylase has a wide range of applications. These applications are mentioned in Chapter 1. Our enzyme is thermostable and alkalostable. Therefore we have attempted to study the application of our enzyme in:

A. Textile desizing

B. Detergents

C. Bioprocessing of starchy wastes

7.1.A. Application of amylase produced by *B. licheniformis* SPT 27 in textile desizing:

Weaving of fabric places considerable strain on the wrap. To prevent breaking of these threads, they are usually strengthened by application
of an adhesive size. Throughout the world the predominant size is still based on starch, despite the introduction of other substances like gelatine, plant gums, water soluble cellulose such as methyl and carboxy methyl cellulose and polyvinyl alcohol.

The economic value of, very inexpensive, starch has maintained its dominance. The type of starch used is really a matter of geographical availability. Europe uses mainly potato starch, the North American textile industry uses maize starch and the Middle and Far East mainly uses rice starch.

Sized cloth is less absorbant. Therefore uptake of dyes, bleaches and texturizing chemicals is impaired unless the size is adequately removed. Many garments, especially are desized after machining.

Desizing is done after singeing. By singeing the cotton goods get smooth surface.

There are three methods of desizing:

a. Rot-steep
b. Acid-steep
c. Enzyme-steep.
Rot steeping is the oldest method of desizing, which depends on the presence of starch liquifying microorganism in air and fabric which multiply in warm water.

All of them depend on breaking down the size preparation until it is soluble in water, but the cellulose must not be attacked during this process. Truly water soluble size can be removed with hot water and detergent washing, but the starch sizes need degrading to make them soluble.

In acid steep, 0.5 to 1% sulphuric acid solution is allowed to act on the size for 4 to 12 hours at 40°C but great care is necessary in order to avoid any attack on the cellulose itself. A thorough rinsing afterwards is essential, for otherwise the cotton would be degraded if the acid was allowed to concentrate on drying.

Enzyme steeping is the most popular and safest method of desizing. These enzymes do not endanger the cotton. Of the various types of enzyme, the malt diastase was most widely used. The best results were obtained with pH of 6.5 and a temperature of 60°C. Pancreatic enzymes are available and are best used at 50 to 55°C in solution of pH 7 to 7.5. These enzymes became costly.
Bacterial enzymes are the best choice. They operate best between pH 5.5 to 7.7 so that they offer more latitude, but the temperature should not exceed 70 °C. One hour is allowed for desizing. With suitable condition bacterial enzyme may desize in 15 minutes.

In far east, starch hydrolyzing enzyme preparation from fermentation products like Saki were used for textile application. These enzyme concentration were obtained from koji process of cultivating mixed fungal population on moist cereal bran and then extracting with minimum amount of water. The important practical limitation to the use of these enzyme are their relatively slow action, for their cost and their sensitivity to the environment in which they are to function. Most of these problems have been solved by introduction of bacterial amylase and by the advent of thermostable amylase.

About 1% on the weight of the goods of amylase is added to the steeping liquor. The cotton is either soaked in this solution at a temperature of 65 °C (149 °F) for several hours or is simply impregnated with warm solution and allowed to stand.
With thermostable enzyme it is possible to accelerate desizing process. They can work at high temperature and are less sensitive to other chemical present in desizing liquor.

Conventional amylase exhibit a sharp decline in performance when desizing temperature is above 75 °C. The performance of thermostable amylase is three times greater at 95 °C than at 60 °C, the temperature at which conventional enzyme shows roughly equal performance.

**Desizing surfactant and enzyme performance:**

To ensure even action of enzyme it is usual to add powerful surfactants to desizing liquors. The starch becomes swollen by hot water and must thoroughly be wetted to ensure that water for hydrolytic action is readily available.

The desizing process involves 4 main stages:

a. Prewashing
b. Impregnation
c. Starch hydrolysis
d. After wash
Scouring is the process which follows desizing. Unlike wool, natural cotton contains comparatively small proportion of impurities. The waxes are of high molecular weight. This makes its removal difficult. The proteins are situated in the central cavity of fiber and are therefore relatively inaccessible to chemical attack.

The object of scouring is to remove oils, fats, waxes, soluble impurities and any particulate or solid dirt adhering to the fibers. The process consists essentially of treatment with detergent with or without the addition of alkali. When soap is used, a good supply of soft water is required. Cellulose is not affected by prolonged boiling with sodium hydroxide solution of concentration up to 2% provided air is excluded. Therefore all the impurities other than natural colouring matter is converted into soluble compound which can be washed away with water.

Boiling with alkali is carried out in specially constructed vessels. These vessels are known as kiers. These may be open, when the liquor boils at atmospheric pressure, or closed when liquor will boil under pressure at temperatures above 100 °C.
Since our enzyme was alkalostable and thermostable, we aimed at trying to do desizing along with scouring i.e. when our enzyme is used for desizing at high temperature and high pH, the final effect of enzyme action should be similar to that of desizing and scouring, so that there would be no need of doing scouring as a separate process.

7.2. A MATERIALS AND METHODS

7.2. A.1 Prewashing :-

The sample is kept in boiling water for 15 minutes

7.2. A.2. Impregnation :-

A bath with enzyme liquor is used. The amount of enzyme, temperature, time etc are varied according to the experiments.

7.2. A.3. Starch hydrolysis:-

Starch hydrolysis is carried at test temperature, time, pH and enzyme load. The fabric is left in the enzyme liquor for starch hydrolysis.

7.2. A.4. After wash:-
The hydrolysed product is removed by washing in water (70 to 80 °C), with agitation.

7.2. A.5. Monitoring starch desizing:

The method is based on the reaction of starch with dilute solution of iodine. With raw starch, the color produced is deep blue black which pales to blue and then to violet as starch is broken down. A pale yellow brown color indicates that all starch has been hydrolysed. The test solution is made to 0.005 N iodine by diluting the stock iodine solution with water at ratio of 1 part stock to 19 parts water.

To monitor the progress of desizing process a portion of cloth is treated with few drops of dilute iodine solution and the color noted after an interval. As the test is sensitive and will be positive even to traces of starch residues, it can be used.

7.2. A.6. Stock iodine solution (0.1 N):

Dissolve 18 g KI and 12.69 g iodine in about 500 ml of water and dilute it accurately to 1 liter. It is stored in brown glass bottle away from light. This stock solution can be kept for many months.
7.2.A.7. Dry weight of the fabric :-

From the sample, take 10 g piece of it. Shred it to bits. Mix well. Take 5 specimen each of 1 g. Check the percentage of moisture of specimen by drying it at 105°C – 110 °C to consistent weight.

7.2.A.8. Preparation of extract:-

Take the test specimen. Weigh it. Boil the specimen in 150 ml distilled water for 45 minutes. Cool it. Decant into beaker. Add 25 ml of distilled water to residue in conical flask. Boil for 30 minutes. Decant it in above the beaker. Put a drop of iodine of the fabric. Observe for blue color. If there is blue color repeat the process. If there is no blue color it means the residue is free of starch. Filter or decant the liquor. Concentrate the filtrate by boiling. Cool to room temperature. Make final volume to 100 ml with distilled water.

7.2.A.9. Starch estimation:-

a 0.5 N, K₂Cr₂O₇ :-

Take 24.5 g K₂Cr₂O₇ (Ranbaxy) and 300 ml of H₂SO₄ (Ranbaxy) (concentrate) and make final volume to 1.0 liter with distilled water.

b Ferrous ammonium sulphate (0.1N) :- Take 39.5 – 40 g ferrous ammonium sulphate (SDfine) and 20 ml of concentrated sulphuric acid (Ranbaxy). Make final volume to 1 liter with distilled water.

c. Indicator :- Take 0.13 g anhydrous sodium carbonate (Ranbaxy) in little water. Dissolve in this 0.26g phenyl anthanilic acid (EMerck) and make final volume to 250 ml.
7.2.A.9. Estimation of starch:-

Take 25ml of extract in 250ml flask. Add 10ml solution A (Potassium dichromic acid) and 5ml of concentrated sulphuric acid. Boil under reflux for 1 hour. Cool it. Final volume is made to 100ml. Titrate this with ferrous ammonium sulphate with 6 drops indicator towards the end of titration. Green color indicates end of titration.

Oven dry weight (g) = \( w \times \frac{m}{100} \)

\( w \) = weight in 'g' of specimen.

\( m \) = moisture content %

\% Starch = \( \frac{400 \times (V1 - V2) \times N \times 0.00696}{W} \)

\( V1 \) = ml of ferrous ammonium sulphate required for blank

\( V2 \) = ml of ferrous ammonium sulphate required for extract

\( N \) = Normality of Ferrous ammonium sulphate

\( W \) = Oven dry weight in 'g' of specimen.

1ml of 1 N, \( K_2Cr_2O_7 \) = 0.00696 g starch

For blank 25ml distilled water is taken instead of extract.

(ISI Handbook of textile testing)
7.3.A RESULTS:-

7.3.A.1 Optimization of time and temperature for desizing:

An enzyme feed of 15000 U was taken for 1 g of grey fabric without any pre wash of the specimen. The process was carried out at 40, 60, 70, 80, 90 and 100°C without addition of any additive or wetting agent.

The residual starch was determined which showed that even after 120 minutes at 40°C, the residual starch was 2.4g%, while at 60°C, 70°C, the residual starch was 0.23g%. The longer incubation time did not improve the desizing effect. At 80°C after 5 minutes, the residual starch was 0.219g% while after 15 minutes the value reduced to 0.109 g% which was unaffected even after prolonging the incubation time at 80°C or by increasing the temperature to 90°C or 100°C (Table 7.1).

7.3.A.2 Effect of water washes during desizing at pH 9, 100°C for 15 minutes:

Results indicate that the washing does not affect the desizing process when done at pH 9, 100°C for 15 minutes. The % of the residual starch under all conditions is 0.1 g.

7.3.A.3. Effect of salts and wetting agent on desizing:

Desizing was carried out at 100°C for 15 minutes and pH 9. Desizing when done in presence of calcium ions showed 0.038 g% residual starch which is better than one done in presence of sodium ion which showed 0.052 g% of residual starch. Desizing when done in presence of calcium ions, sodium ions along with wetting agent gave the best results (0.013 g% of residual starch) while desizing done with wetting
### TABLE 7.1

**EFFECT OF TEMPERATURE AND TIME ON DESIZING**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time (minutes)</th>
<th>9% residual starch/gram fabric</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>120</td>
<td>2.4</td>
</tr>
<tr>
<td>60</td>
<td>120</td>
<td>0.232</td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>360</td>
<td>0.22</td>
</tr>
<tr>
<td>70</td>
<td>15</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.23</td>
</tr>
<tr>
<td>80</td>
<td>5</td>
<td>0.219</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.109</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.109</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.109</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.109</td>
</tr>
<tr>
<td>90</td>
<td>5</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.11</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
<td>0.1</td>
</tr>
</tbody>
</table>
agent with either calcium or sodium ion was not better than when done in combination (Table 7.2)

7.3.A.4. Effect of enzyme loading on desizing :-

The desizing was done at 100°C for 15 minutes using different concentrations of enzyme. The effect of desizing had improved to some extent but than was not affected with any further increase in enzyme units (Table 7.3).

7.3.A.5. Effect of surfactant additives on desizing at 100°C for 15 minutes at pH 9.0:-

The results indicate that the Tween 60 was more efficient than Tween 20. The desizing in presence of Lissapol N was the best compared to desizing in presence of other additive. Desizing could be carried out efficiently even in presence of EDTA (Table 7.4)

7.4. A General Discussion

Our aim was to see if our enzyme could be used for desizing at higher pH. After desizing , a process called scouring is done. The object of scouring is to remove oils, fats, waxes, soluble impurities and particulate matter adhering to the fiber.

Scouring of cotton is done by boiling with sodium hydroxide of concentration upto 2%. This makes it possible to change all the impurities other than natural colouring matters into soluble compounds which can be washed away with water. The amylase produced by B. licheniformis SPT-27 is stable at pH 10 and had showed activity at pH
### TABLE 7.2

**EFFECT OF SALTS/WETTING AGENT ON DESIZING**

<table>
<thead>
<tr>
<th>Additives</th>
<th>% Residual starch/g fabric</th>
<th>% residual amylase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium chloride (8mg%)</td>
<td>0.038</td>
<td>63</td>
</tr>
<tr>
<td>Calcium chloride (80Mm)</td>
<td>0.038</td>
<td>83</td>
</tr>
<tr>
<td>Sodium chloride (8mg%)</td>
<td>0.052</td>
<td>83</td>
</tr>
<tr>
<td>Wetting agent (WA)</td>
<td>0.026</td>
<td>80</td>
</tr>
<tr>
<td>WA + NaCl (8mg%)</td>
<td>0.06</td>
<td>60</td>
</tr>
<tr>
<td>WA + calcium chloride (80mM)</td>
<td>0.065</td>
<td>60</td>
</tr>
<tr>
<td>WA + CaCl₂ + NaCl</td>
<td>0.013</td>
<td>55</td>
</tr>
<tr>
<td>Control</td>
<td>0.32</td>
<td>50</td>
</tr>
</tbody>
</table>

**DESIZING AT 100°C/15MINUTES/pH 9**
### TABLE 7.3

**EFFECT OF ENZYME LOADING ON DESIZING**

Desizing conditions: pH 9/15 minutes/100°C

<table>
<thead>
<tr>
<th>ENZYME CONCENTRATION (IU)</th>
<th>9% RESIDUAL STARCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>5000</td>
<td>0.5</td>
</tr>
<tr>
<td>10000</td>
<td>0.25</td>
</tr>
<tr>
<td>15000</td>
<td>0.103</td>
</tr>
<tr>
<td>30000</td>
<td>0.103</td>
</tr>
<tr>
<td>45000</td>
<td>0.103</td>
</tr>
</tbody>
</table>
TABLE 7.4

EFFECT OF SURFACTANTS ADDITIVES ON DESIZING

<table>
<thead>
<tr>
<th>Surfactants Additives</th>
<th>% residual starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium lauryl sulphate</td>
<td>.075</td>
</tr>
<tr>
<td>Tween 20</td>
<td>.068</td>
</tr>
<tr>
<td>Tween 80</td>
<td>.024</td>
</tr>
<tr>
<td>Triton</td>
<td>.036</td>
</tr>
<tr>
<td>Lissapol N</td>
<td>.015</td>
</tr>
<tr>
<td>EDTA</td>
<td>.031</td>
</tr>
<tr>
<td>Sodium perborate tetrahydrate</td>
<td>.031</td>
</tr>
<tr>
<td>Tn sodium pyrophosphate</td>
<td>.028</td>
</tr>
<tr>
<td>Sodium tripolyphosphate</td>
<td>.017</td>
</tr>
</tbody>
</table>

Desizing conditions: 100°C/pH 10/15 minutes
12 also. Therefore we had carried out desizing at pH 10 and found that
the process was efficient.

Detergents are surface active compounds and these are also used in
textile industries to remove all the impurities, leaving the fibers in such
a state that they are equipped for the purpose for which they are
intended. Soap has now, to a very great extent, been replaced by
anionic or nonionic synthetic detergents as they are cheaper than soap
and avoids the expense of softening water. Synthetic detergents do not
react with calcium and magnesium salts in hard water.

Detergents are good wetting agents. The primary alkyl sulphate could
be used as detergent. Lauryl sodium sulphate (Gardinol WA) is an
anionic detergent and the enzyme was active to desize leaving,
0.075% residual starch on the grey fabric.

Lissapol N is a nonionic surface active compound and is the
condensation product of on nonyl phenol with ethelene oxide. They are
suitable for scouring even at low temperature. The desizing was best
achieved in presence of Lissapol N. The amylase enzyme was active in
presence of bleaching agents and had carried out quite successfully
the desizing process.

The detergents act as wetting agents, thus giving better desizing
effects. The wetting agent assures the even action of enzyme on grey
cloth while the ions help in improving the enzyme stability. Robinson
(1937) showed that simple electrolytes like sodium salts lower the
interfacial tension between sodium alkyl sulphate and oils. Addition of
such electrolytes therefore improves detergency.
On carrying out desizing of the grey fabric, which contains 3.5g% starch, with alpha amylase produced by *B. licheniformis* SPT-27, at 100°C and pH 9 and 12 for 15 minutes, the residual starch of the fabric was 0.02 g% and 0.015 g%, respectively. This indicates that there was a removal of 99.57% of starch size from the fabric and there was a scouring effect too.

In order to understand the changes on the surface of the fabric, scanning electron microscopy of the fabric desized at 100°C, pH 12 for 15 minutes was done. The electronmicrograph showed that the size on the fibers were hydrolysed as the surface of the fabric was softer, brighter and more absorbant (fig 7.1).

7.1.B Application of alpha amylase produced by *Bacillus licheniformis* SPT 27 in detergents

Detergency means cleansing. It is the removal of undesirable or foreign substance from required material. There are many factors involved in mechanism of detergency and the relative importance is influenced by nature of dirt. The lowering of surface tension at oil-water interface accompanied by formation of easily detached sphenical globules is the essential mechanism of removing oil. When the dirt is amphiphilic the soap molecule concentrates at the interface where they form tertiary water-amphilic dirt\soap system. On saturation it forms highly viscous liquid crystal membrane. Penetration of surrounding solution of soap in water through membrane forces out micelle which disperse into surrounding detergent liquid to form an isotopic phase.
Detergents are used for domestic washings and laundries. The pH of the detergents are generally alkaline while in use. The efficiency of detergents of removing proteinaceous and oil stain has been improved by addition of protease and lipase enzyme. These are called "biological detergents" and are the products of biotechnology industry.

Amylase can be incorporated in the detergents which will remove starch based stains. They will ease the removal of protein bound starch stains by working synergistically with proteinases.

Most commonly used enzymes in detergent formulation are proteinases and amylases. Lipase and cellulase have recently been introduced, but their application is more controversial. Consumers were afraid of possible allergic reaction to proteolytic enzyme added to detergents. Recently however the situation has changed due to the fact that decreased oil consumption has become of interest. Consumers are demanding low price detergents. No allergic reaction have been reported after lengthy experiments, to alkaline enzyme detergent additives. Some of the enzyme added in detergents are Biotex, Termamyl, Maxamyl which are effective up to 100°C and showing good performance up to pH 10. (Information Brochure and datasheet. International Biosynthesis B.V, Rijswijk. The Netherlands).

It has been customary to add various alkaline salts to improve the detergency of soap. A builder may be defined as a compound which has no surface active properties but which increases the efficiency of detergent. Builders like sodium carbonate, sodium bicarbonate gives mildly alkaline conditions which would neutralize the acidic dirt.
Phosphates are commonly added as builders, sequesters calcium, magnesium and other metallic ions.

The success of alkaline amylase in detergents is dependent on their possession of the following characters:

- wide pH activity range,
- stability under highly alkaline conditions,
- high activity and stability in presence of surfactants,
- stability in presence of builders,
- high activity over a wide temperature range,
- good solubility,
- good storage stability

The composition of the basic detergent is given in Table 7.5

7.2.B. Materials and Methods

7.2.B.1. Stability test of alpha amylase in presence of commercial detergents:

25 g/ml of commercial detergent powder was taken with crude alpha amylase. The pH was 10. The tubes were incubated at 37°C, 60°C and 100°C for 60 minutes in presence of calcium ions (25mM).
<table>
<thead>
<tr>
<th>Components</th>
<th>g%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear sodium alkylbenzene</td>
<td>11</td>
</tr>
<tr>
<td>Nonyl phenol ethoxylate</td>
<td>2.5</td>
</tr>
<tr>
<td>Sodium soap</td>
<td>3.0</td>
</tr>
<tr>
<td>Carboxymethylcellulose sodium salt</td>
<td>1</td>
</tr>
<tr>
<td>Tetra sodium EDTA</td>
<td>.18</td>
</tr>
<tr>
<td>Sodium triphosphate</td>
<td>38</td>
</tr>
<tr>
<td>Sodium perborate tetrahydrate</td>
<td>25</td>
</tr>
<tr>
<td>Sodium silicate</td>
<td>7</td>
</tr>
<tr>
<td>Sodium sulphate</td>
<td>100</td>
</tr>
</tbody>
</table>
7.3.B. Results:-

7.3.B.1 Effect of detergents on amylase stability:-

The enzyme retained all the activity when incubated with detergents at 37°C and 60°C while at 100°C it retained 55% of residual activity (Table 7.6).

7.4.B GENERAL DISCUSSION :-

Earlier studies on the enzyme's thermostability in presence of various additives which are generally used as ingrediants in the detergents, has shown that the enzyme had retained most of the activity. In addition, when used with commercial detergents, the enzyme then too, had retained good amount of activity even at 100°C. This means, that our enzyme can be used and included in detergents.

Due to energy saving consciousness and increased use of more delicate synthetic fabric, washing time are continuously decreasing. The demand for proteinases, amylase, lipase, cellulase has increased to compensate for decreased washing performance at lower temperature.

The detergent market is very segmented due to widely differing consumer practices which determine the use of detergent and their formulation. Americans use a short washing cycle. The water is added at 50°C and allowed to cool during a 5 to 15 minutes wash at 20 to 30°C (Kalisz, 1988; Brenner, 1987). European washing condition involve warming the water to 40 or 60°C in a 30 to 60 minutes cycle with or without a cool prewash (Kalisz, 1988; Winkhans, 1987).
## Table 7.6

**Effect of Detergents on Amylase Stability at Different Temperatures (1 Hour)**

<table>
<thead>
<tr>
<th>Detergents (2.5g%)</th>
<th>37 °C % residual activity</th>
<th>60 °C % residual activity</th>
<th>100 °C % residual activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerial</td>
<td>100</td>
<td>100</td>
<td>55</td>
</tr>
<tr>
<td>Wheel</td>
<td>100</td>
<td>100</td>
<td>57</td>
</tr>
<tr>
<td>Nirma</td>
<td>100</td>
<td>100</td>
<td>55</td>
</tr>
<tr>
<td>Jyoti</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Rin</td>
<td>100</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>Rin (white)</td>
<td>100</td>
<td>100</td>
<td>59</td>
</tr>
</tbody>
</table>
The alpha amylase produced by *B. licheniformis* SPT–27 is efficient at 37°C and 60°C so can be used for washing at both the temperatures (Grant et al., 1990)

7.1.6 Application of alpha amylase from *B. licheniformis* SPT–27 for bio processing of starchy wastes:

There is an increasing awareness of pollution created by wastes and the need to recover some valuable resources in reuse. The bioprocessing of waste must be integral and of viable economic activity.

Starch processing waste materials are produced from food processing plants, cold storages of vegetable and domestic waste. Biotechnological treatment of food-processing wastes can produce valuable products while also purifying the effluent. (Bergman et al., 1988; Friendrich et al., 1987; Jamuna and Ramakrishna, 1989, Klingspohn et al., 1993, Sukan and Yasin, 1986).

In most of the works reported earlier, the material used as substrate had to be pretreated for sterilization and partial hydrolysis of starch to sugar. Aseptic operation condition and longer retention time were required to carry out the process. These increases, the process cost and are uneconomical.
7.2.C. MATERIALS AND METHODS:-

7.2.C.1. Substrate :-

Starchy industrial waste - water from bakery industry were used as such. The raw potato which got spoiled during storage and the domestic potato waste were also taken for studies.

7.2.C.2. Cultural conditions :-

50 ml of the bakery industrial starchy effluent was taken in 250 ml of Erlenmeyer flask. 1 % of 8 hour old inoculum was taken. The flasks were incubated on the shaker for different period of time at 37°C. The activity was estimated by DNS method.

In case of raw potato, 250 mg of raw unsterile domestic potato waste were taken in 1 liter flask. To this 10 ml of 8 hour old *B. licheniformis* SPT - 27 grown in nutrient broth was inoculated. The flask was incubated at 37°C at static conditions. Two such flask were taken. One flask had a pH of 12 and the other had a pH of 7. Rest of the cultural conditions were same.

7.3.C. RESULTS:-

7.3.C.1. Amylase activity in bakery industrial effluent:-

There was no significant amylase activity even after 72 hours of incubation.
7.3.C.2. Amylase activity in potato waste at different pH:

A good amount of amylase activity was observed at pH 12. At neutral pH the activity was observed to be very less and there was fungal contamination. (Table 7.7).

7.3.C.3. Amylase activity in potato waste at different temperatures:

The maximum amylase activity was detected at 30°C and 45°C after 24 hours incubation in static condition while at 20°C the activity observed after six days was 40% of the activity observed at other two temperatures at static conditions. (Table 7.8)

7.4.C. GENERAL DISCUSSION:-

The domestic potato waste in form of potato peels, or potatoes which get spoiled due to physical injury during storage by piling or during storage in refrigerators can be used as substrate for amylase production. Due to the absence of pretreatment and nutrient supplementation together with non aseptic operation conditions the process is economically attractive.

The operating costs are reduced because the *B. licheniformis* SPT –27 can grow at pH 12 thus eliminating the contaminants.

The process is technically feasible for the treatment of raw potato waste in cooler and warmer climates. Ghai et al. (1980) had isolated *Aspergillus niger* F 51 and used for alpha amylase production using low grade potatoes as substrate.
<table>
<thead>
<tr>
<th>pH</th>
<th>12</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hour</td>
<td>Amylase activity (IU/ml)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>112</td>
</tr>
<tr>
<td>12</td>
<td>126</td>
<td>140</td>
</tr>
<tr>
<td>18</td>
<td>213</td>
<td>140</td>
</tr>
<tr>
<td>24</td>
<td>280</td>
<td>140</td>
</tr>
</tbody>
</table>

TABLE 7.7

AMYLASE YIELDS WITH POTATO WASTE AT pH 12 & 7
TABLE 7.8

AMYLASE ACTIVITY ON POTATO WASTE AT DIFFERENT TEMPERATURE

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Amylase activity(Units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>336</td>
</tr>
<tr>
<td>45</td>
<td>332</td>
</tr>
</tbody>
</table>

Incubation conditions: 24 Hours/ pH 10/ static