# EFFECTS OF pH ON GLUCOAMYLASE PRODUCTION BY *Aspergillus awamori* NRRL 3112 IN AN AIRLIFT FERMENTER

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## ABSTRACT

**The effects of pH on batch production of glucoamylase by *Aspergillus awamori* NRRL 3112 in a 3.5-L airlift fermenter were determined using a mixture of cassava flour and rice bran (1:2 weight ratio) as substrate. Highest production of the enzyme was noted on the fourth day of incubation when the pH of the medium was maintained at 5.5. The volumetric and specific activities of glucoamylase were 124.4 IU/mL and 10.3 IU/mg protein, respectively.**

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**KEY WORDS:** Glucoamylase. *Aspergillus awamori* NRRL 3112. pH. Airlift fermenter. Batch production. Cassava flour. Rice bran.

## INTRODUCTION

Glucoamylase (α-1,4 glucan glucohydrolase, E.C. 3.2.1.3) is a carbohydrase which hydrolyzes starch, glycogen, malto-oligosaccharides and similar compounds almost completely to D-glucose. In recent years, glucoamylase has assumed considerable importance because of its ability to hydrolyze starch to glucose with little formation of undesirable products as compared to acid hydrolysis (Smiley et al., 1971). Fungal glucoamylase may be used in the production of ethanol from starchy materials, in bread-making to improve the quality of bread and in the production of dextrose.

Glucoamylase is produced by various species of *Aspergillus* and *Rhizopus* as well as by some yeasts and bacteria. One of the critical

factors that affect its production is the hydrogen-ion concentration or pH of the medium. This study thus aimed to determine the influence of different pH levels on glucoamylase production by *Aspergillus awamori* NRRL 3112 employing a mixture of cassava flour and rice bran as substrate.

## MATERIALS AND METHODS

*Microorganism. Aspergillus awamori* NRRL 3112 was obtained from the Northern Regional Research Center, U.S. Department of Agriculture, Peoria, Illinois, U.S.A. The microorganism was grown on potato dextrose agar (PDA) slants at room temperature (29°C) for 6 to 7 days. Spore suspension was obtained by adding 5 mL sterile distilled water into each slant and rubbing the surface gently with sterile wire loop. The suspension containing approximately $10^7$ spores per mL was used in the inoculum build-up. The number of spores was estimated using the haemacytometer method as described by Colle et al. (1982).

*Inoculum Build-up.* Ten percent (w/v) solids level of substrate consisting of cassava flour (100 mesh) and rice bran (100 mesh) in a 1:2 weight ratio was used. The mixture was treated with 0.4N sulfuric acid (1 mL $H_2SO_4$/g solids) and was heated at 75-80°C for 15 minutes with constant stirring. After cooling, the pH was adjusted to 4.5 with 2N ammonium hydroxide ($NH_4OH$). The desired volume (350 mL) was taken, sterilized at 121°C for 15 minutes and cooled to room temperature. The medium was inoculated with 35 mL spore suspension and incubated at room temperature for 24 hours with continuous shaking.

*Medium for Glucoamylase Production.* The same substrate constituents and weight ratio as in the inoculum build-up was used for glucoamylase production. The desired amount (700 g) of substrate was treated with 0.4N sulfuric acid (4 mL $H_2SO_4$/g solids) and heated at 75-80°C for 15 minutes with constant stirring. After cooling to room temperature, 6N $NH_4OH$ was added (volume of base = 1/20 x volume of acid). While the medium was diluted with tap water to obtain 20% (w/v) solids level, the pH was at the same time adjusted to the desired level with 6N $NH_4OH$. The medium was placed in a suitable container, sterilized at 121°C for 15 minutes and cooled to room temperature.

*Glucoamylase Production.* Glucoamylase was produced at three pH levels, namely: 5.0, 5.5 and 6.0. The medium together with 10% (v/v) inoculum was pumped into a sterilized 3.5-L stainless steel airlift fermenter using a peristaltic pump. Incubation was carried out at 30 ± 0.2°C for 5 days with an aeration rate of 0.8 to 1.0 volume of air per volume of medium per minute (vvm). The aeration rate was measured with a Gilmont flowmeter. The desired temperature and pH were maintained by means of a temperature controller (YSI Model 73 ATC)

and a pH controller (New Brunswick Model), respectively. The pH was controlled by automatic addition of either 6N $NH_4OH$ or 2N $H_2SO_4$. A few mL of the cultivation medium was taken every 24 hours, centrifuged at 5000 rpm for 20 minutes, filtered and then assayed for glucoamylase activity.

*Glucoamylase Assay.* Glucoamylase activity was determined using the method of Bachler et al. (1970). Four and one-half mL of previously boiled 4% soluble starch solution in 0.05M sodium acetate buffer (pH 4.2) was placed in a test tube and equilibrated at 40-42°C in an Eberbach water bath shaker. One-half mL of suitably diluted enzyme solution was added to the test tube which was then stoppered with marble and incubated for exactly one hour at 40-42°C. Enzyme action was stopped by placing the reaction mixture in boiling water bath for 20 minutes. Reducing sugar formed was measured by the dinitrosalicylic acid (DNS) method as modified by Miller (1959) using glucose as standard. The protein content of the enzyme solution was determined using the procedure of Lowry et al. (1951) with bovine serum albumin (Sigma) as standard. Glucoamylase volumetric activity was expressed as IU per mL and specific activity as IU per mg protein. One international unit (IU) of enzyme activity is the amount of enzyme which produces one micromole of reducing sugars (expressed as glucose) per minute.

## RESULTS AND DISCUSSION

The volumetric activities of glucoamylase produced by *A. awamori* NRRL 3112 as affected by variation in pH are shown in Figure 1. At pH 5.0 and 5.5, the enzyme was produced increasingly until the fourth day of incubation. Maximum enzyme production at both pH levels was noted on the fourth day when the volumetric activities reached 88.0 and 124.4 IU/mL, respectively. In general, microbial enzymes appear to be elaborated after extended incubation. The synthesis of glucoamylase at shorter incubation period may be related to the organism's ability to utilize the smaller nitrogenous compounds such as the amino acids found in rice bran (Kik, 1956).

Figure 2 shows the protein levels in the cultivation medium during the course of incubation. The increasing protein levels are due to the increasing concentration of glucoamylase which is an enzyme, hence also a protein. At pH 5.0 and 5.5, protein concentrations reached the maximum values which were 12.4 and 12.0 mg/mL, respectively on the fourth day. The relatively low protein levels at pH 6.0 indicate that the environment was less favorable either for growth or enzyme synthesis of the mold.

High specific activities of glucoamylase observed at pH 5.5 (Fig. 3) demonstrate that glucoamylase synthesis is enhanced at this pH value or that the enzyme is more stable at

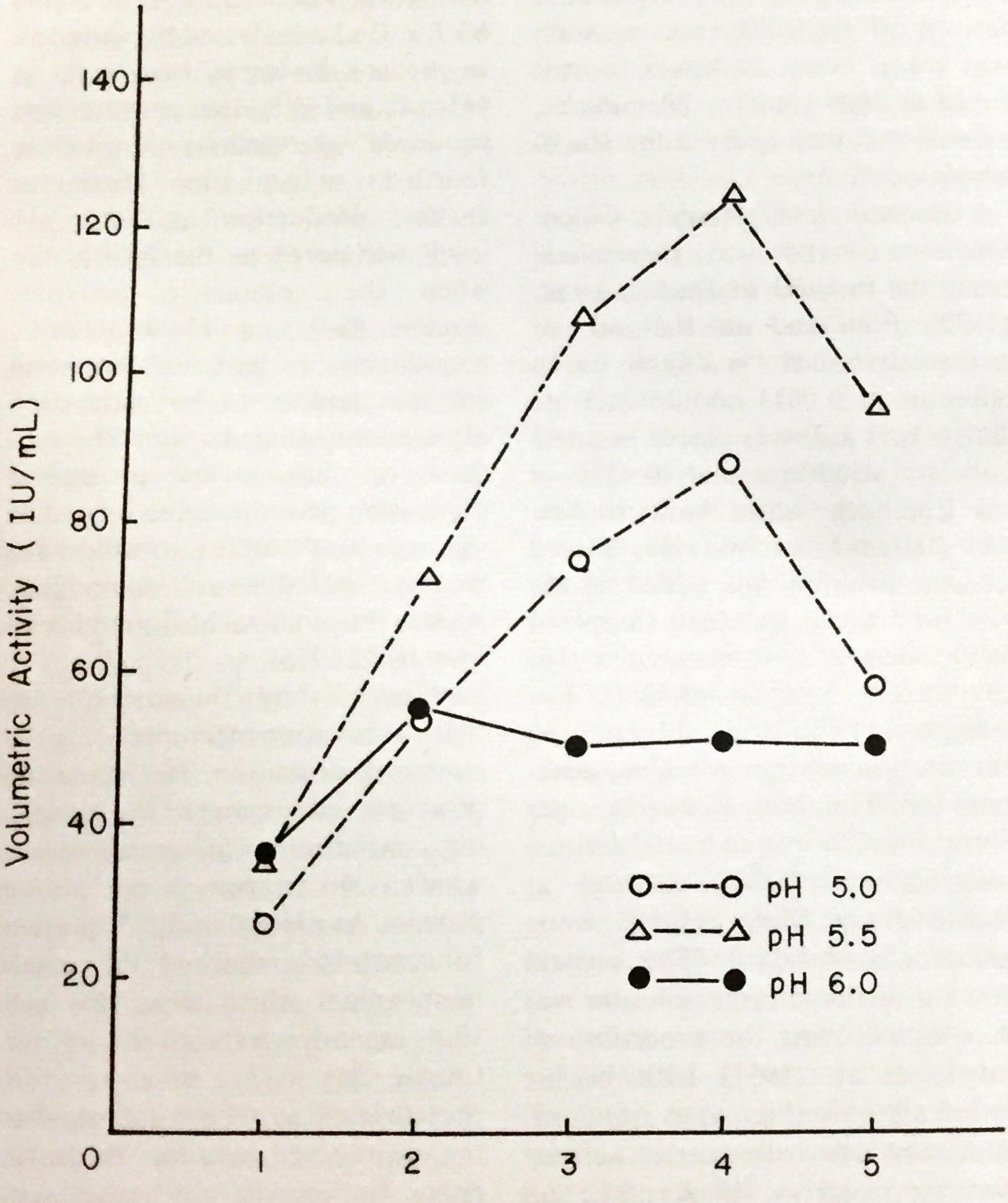


**Figure 1.** Volumetric activity of glucoamylase produced at different pH.

this pH level. As Nithiandam et al. (1981) and Osorio (1981) noted, soluble glucoamylase has maximum stability at pH 5.5. Furthermore, Davies (1963) reported that the greatest yield of most extracellular

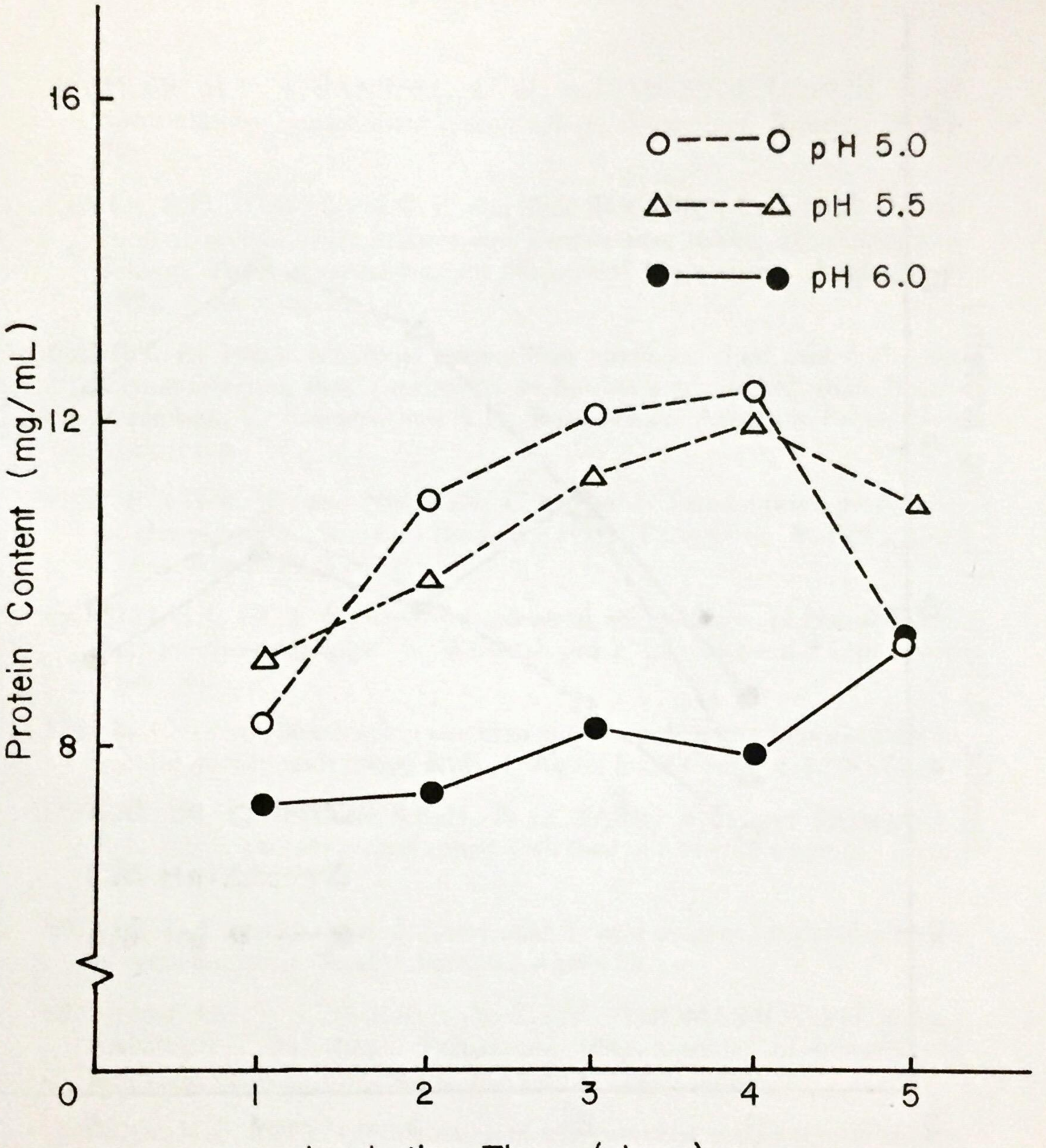


**Figure 2.** Protein content of the solid-free enzyme extract during production of glucoamylase at different pH.

enzymes is obtained at a growth pH somewhere near the pH for maximum activity. The decrease in volumetric and specific activities could be explained by the fact that during prolonged incubation, microorganisms tend to produce proteases thus, inactivating the desired enzyme (Gould, 1975).

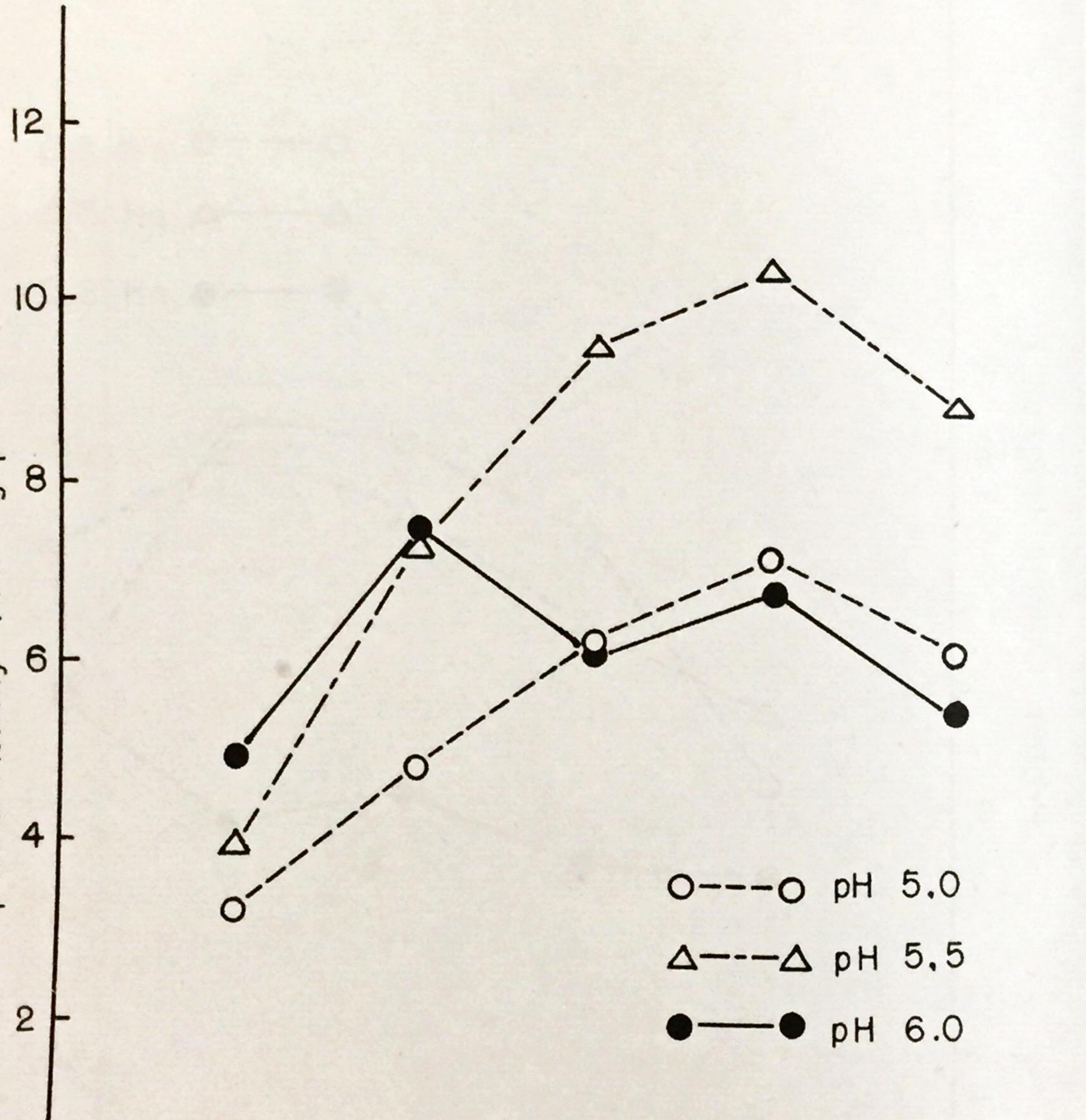


**Figure 3.** Specific activity of glucoamylase produced at different pH.

The above results confirm the findings of other workers. Osorio (1981) mentioned that the optimum pH for shake-flask production of glucoamylase by the same mold is 5.5. Large-scale production of the enzyme by the same microorganism at pH 5.5 was also reported by Smiley et al. (1964) and Dworzchak and Nelson (1972).

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