ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/289435051

Production of α-amylase in a corn steep liquor-soya bean meal medium by a strain of Bacillus stearothermophilus

Article · January 1997

CITATIONS
5

READS
49

4 authors, including:

Olusesan Omidiji
University of Lagos
24 PUBLICATIONS **1,011** CITATIONS
SEE PROFILE

Olukayode Amund
University of Lagos
77 PUBLICATIONS **689** CITATIONS
SEE PROFILE

Matthew Olusoji Ilori
University of Lagos
73 PUBLICATIONS **959** CITATIONS
SEE PROFILE

Available from: Olukayode Amund
Retrieved on: 23 May 2016

Production of α-amylase in a corn steep liquor-soya bean meal medium by a strain of *Bacillus stearothermophilus*

O. Omidiji, O. O. Amund, A. A. Braimoh and M. O. Ilori*

Department of Biological Sciences, University of Lagos, Yaba, Lagos, Nigeria
(*Reprint address)

Key words: amylase, corn steep liquor, soya bean, *Bacillus stearothermophilus*, starch, protein

Abstract

Corn grains (*Zea mays*, yellow variety) were steeped in water for 10 days and parameters such as pH, protein, amino acids, lipid, soluble starch and reducing sugar content were measured at 2 day intervals in the steep liquor. The pH decreased from 7.0 to 5.4 during the steeping period. The protein content ranged between 1.25 and 11.25 mg/ml; lipid content ranged between 0.3 and 3.2 mg/ml. The soluble starch concentration increased from 212 to 530 mg/ml, and the reducing sugar concentration reduced from 2.54 to 1.13 mg/ml. A fermentation medium was compounded from 2-day-old liquor to cultivate an amylolytic strain of *Bacillus stearothermophilus*. Peak production of amylase occurred at 240 h while the highest cell density occurred at 144 h. Various concentrations of soluble starch (0.5–5%, w/v) and soya bean meal (0.1–0.5%, v/v) were used in cultivating the organism. The highest amylase activity was recorded with 2% (w/v) starch, while 0.5% (v/v) of soya bean meal supported the greatest growth. The medium could be exploited for industrial amylase production.

Introduction

A detailed investigation is often required in order to establish the most suitable medium for any fermentation process (Stanbury and Whitaker, 1984). Fermentation media can be prepared using pure substrates which are relatively simple but sufficiently rich to support microbial growth in a small scale process. However, such formulation may be unsuitable for use in a large scale process (Okafor, 1987). The composition of a growth medium for commercial production of microbial products should be adequately balanced to keep the pH within the appropriate limit during fermentation while at the same time ensuring consistent production of the desired metabolites.

Corn steep liquor, a by-product of starch production from corn during soaking was shown to be very rich in the nutrients required by most micro-organisms (Akinrele, 1970; Foda *et al.*, 1973). Its composition was investigated and found to vary depending on the corn variety, steeping conditions and age of the corn kernels (Okafor, 1987). Media formulation using corn steep liquor is a widely practised commercial undertaking arising from the relative cheapness and consistency of such media under standard industrial conditions.

Published and © 1997 by The Faculty Press
88 Regent Street, Cambridge, Great Britain

During industrial processes utilizing corn steep liquor, the ability to generate corn starch simultaneously constitutes an additional asset after removal of the valuable liquor for microbial cultivation.

The study reported in this paper was aimed at formulating an appropriate medium for the growth of a strain of *Bacillus stearothermophilus* and to assess the optimal conditions for the extracellular production of amylase by this bacterium with a view to extrapolating the results obtained to a large scale enzyme fermentation process.

Materials and methods

Bacterial strain

Bacillus stearothermophilus BS31, an α-amylase producing strain, was obtained from the culture collection of the Department of Biological Science, University of Lagos. A freeze-dried culture of the organism was resuscitated and maintained on nutrient agar slants which were stored by refrigeration at 4°C.

Raw materials

A yellow variety of corn grains (*Zea mays*) and soya bean grains (*Glycine max*) were purchased from Adebayo Market, Shomolu, in the Lagos metropolis. Pure corn starch was also obtained in 500 g packets from a department store. Fresh corn and soya bean grains were sun-dried and stored under cool and dry conditions pending use.

Steeping of corn grains

The maize grains were washed thoroughly in tap water and soaked in distilled water (30 g/100 ml) in a kilner jar (1 litre capacity) and kept at room temperature. Samples of steep liquor were withdrawn every 48 h over a period of 10 d for physicochemical and nutrient analyses.

Total sugar assay

The anthrone method of Yemn and Willis (1964) was adopted to assay the total sugar produced. Freshly prepared anthrone reagent (4.0 ml) was added to aliquots (1.0 ml) of corn steep liquor, and the mixture was heated in a water bath (90–100°C) for 10 min. On cooling, the absorbance of the mixture was read at 620 nm in a digital Zeiss spectrophotometer against a reagent blank containing 1.0 ml of distilled water in place of the corn steep liquor. The total sugar content was extrapolated from a standard calibration curve.

Reducing sugar assay

The arsenomolybdate reagent method of Somogyi (1945) was employed for the reducing sugar assay. The reducing sugar content was extrapolated from a standard calibration curve.

Protein assay

Iodine-potassium iodide solution (0.1 ml) was added to corn steep liquor (1.0 ml) and the mixture diluted with 10 ml distilled water. The absorbance was read at 620 nm against a reagent blank (Murata *et al.*, 1968) and the starch content was extrapolated from a standard calibration curve established with soluble corn starch (Sigma, London).

Amino acid assay

The total free amino acid was determined by a modified method of Keay and Wildi (1970) using L-tyrosine as standard.

Lipid assay

The lipid content of corn steep liquor was measured by determining the acid value of fat. One drop of phenolphthalein solution was added to 10 ml of corn steep liquor and the mixture titrated against potassium hydroxide solution (0.1 M) until a faint pink colour persisted. The volume of KOH required to neutralize the mixture was used to estimate the lipid content.

Total acidity

The total acidity was assayed using the method of Ikenebomeh *et al.* (1986). The volume of NaOH (0.1 M) required to neutralize the titratable acids was used to calculate the total acidity.

Total solids

The total solid content of corn steep liquor was measured by evaporating 5 ml of the liquor in a pre-weighed crucible at 80°C in an oven to constant weight.

Fermentation medium

The total fermentation medium had the following components. Two days corn steep liquor (100 ml); soya bean powder (0.2 g), soluble starch (1.0 g), Na_2 HPO_4 (0.1 g) and $CaCl_2$ $2H_2O$ (5 mM). The medium was adjusted to a pH of 7.2, autoclaved at 121°C for 15 min, inoculated on cooling with the test bacterial strain and incubated with shaking (*100 rev/min) at 44°C. Culture supernatants extracted at a 24 h interval were subjected to starch measurement, plate counts and enzyme assays.

Effects of variations in starch and soya bean concentrations

The effects of variation in the concentrations of corn starch (0.0–5.0% w/v) and soya bean meal (0.0–0.5%, w/v) on amylase production and growth of the organism, respectively, were investigated.

Amylase assay

The amylase activity in the culture broth was measured when there was no more starch in the medium (negative iodine reaction). A crude enzyme preparation was obtained by centrifuging 5 ml of broth at

5,000 x *g* and the clear supernatant was used for the assay. Amylase activity was determined by a modified method of Murata *et al.* (1968). The assay tube contained (in duplicate) sodium phosphate buffer (0.1 ml, 0.05 M, pH 6.5), soluble starch (0.1 ml, 0.5% w/v) and the enzyme extract (0.1 ml). The contents were mixed and incubated at room temperature (28 ± 2°C) for 10 min after which the reaction was stopped by the addition of iodine-potassium iodide reagent (0.1 ml). The mixture was diluted to a volume of 10 ml with distilled water. The optical density of the resulting mixture was read at 620 nm against a reagent blank. A second assay tube at the zero time point was also prepared. The reaction in this tube was stopped immediately the enzyme solution was added by the simultaneous addition of the iodine reagent and the mixture diluted with distilled water to a final volume of 10 ml. Amylase activity was expressed as the amount of (mg) starch broken down per ml of enzyme in 1 min.

Results and discussion

Amylase is an important industrial enzyme used for brewing and food processes in Nigeria. Efforts towards local production of the enzyme must include development of suitable amylolytic strains and growth media. Several amylolytic strains with industrial potentials have been isolated and characterized (Amund and Ogunsina, 1987). However, very few studies have been carried out on media formulation for industrial exploitation.

Table 1 Nutrient composition of corn steep liquor obtained from the yellow variety of corn (*Zea mays*)

Parameters	Soaking period (days): 0	2	4	6	8	10
pH	7.0	5.9	5.6	5.6	5.5	5.4
Total acidity		0.26	0.69	0.81	2.44	2.88
Acid value (g/ml)		0.24	0.48	0.66	1.80	2.16
Soluble starch (mg/ml)	0	212	318	371	424	530
Reducing sugar (mg/ml)	0	2.54	2.02	1.65	1.50	1.13
Protein (mg/ml)	0	1.25	2.97	5.63	6.56	11.25
Amino acid (mg/ml)	0	0.30	0.60	1.70	2.10	3.20
Starch recovery after steeping (g/30 g of corn)		5.7	6.6	6.8	7.0	10.7

Table 2 Growth and amylase production by *B. stearothermophilus* in corn steep liquor-soya bean meal medium

Time (h)	Enzyme activity (unit/ml) x 10^3	Total viable count (CFU/ml) x 10^7	Residual starch (%)
0	0	0.5	100
24	0	1.0	98
48	0	1.5	94
72	0	2.0	75
96	6	3.5	62
120	8	7.8	56
144	10	8.0	52
168	14	7.9	48
192	25	7.8	46
216	27	7.4	40
240	54	5.6	7
264	18	4.2	5

The initial (100%) starch concentration was 312 mg/ml.

The effect of the steeping period on some of the parameters needed to formulate an appropriate medium for enzyme production using corn steep is shown in Table 1. There was an increase in soluble starch concentration during steeping of corn while the reducing sugar content decreased simultaneously. The decrease in the concentration of the reducing sugar was probably due to its utilization for growth by the test micro-organism (Akinrele, 1970).

The pH and the total acidity increased with the steeping period. The carboxylic acids produced in corn steep liquor are known to include lactic, acetic, butyric and propionic acids (Plahar and Leung, 1982). The acids produced by the organism soften the membrane of the maize to release its content into the liquor (Okafor, 1987). This may account for the increase in both soluble starch present in the liquor and recovered starch from the softened kernels. The lipid content of the liquor also increased with the steeping period. The average lipid content of edible beans, *Phaseolus vulgaris* L., was 2.2 g per 100 g dry weight (Drumm *et al.*, 1990) while the steep liquor of the yellow maize variety had an average lipid content of about 24.4 g

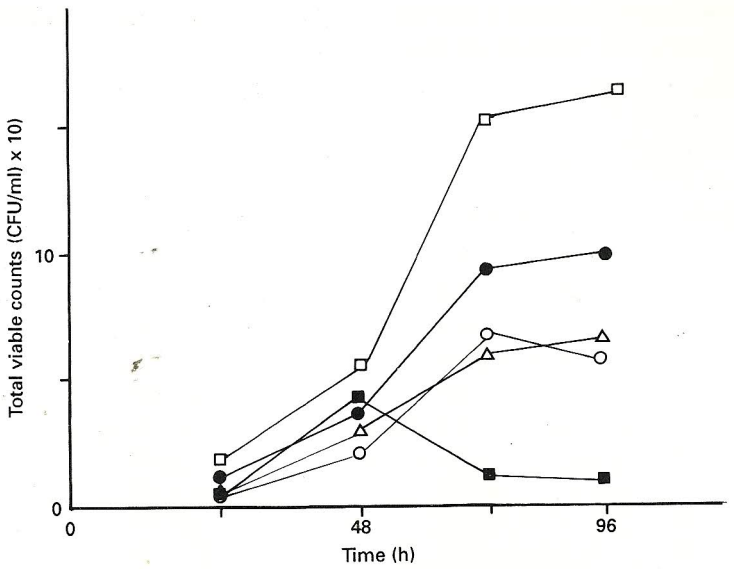

Figure 1 Effect of soya bean concentration on growth of *B. stearothermophilus* in corn steep liquor. ■, No addition of soya bean; △, 0.1% (w/v) of soya bean meal; ○, 0.2% (w/v) of soya bean meal; ●, 0.3% (w/v) of soya bean meal; and □, 0.5% (w/v) of soya bean meal.

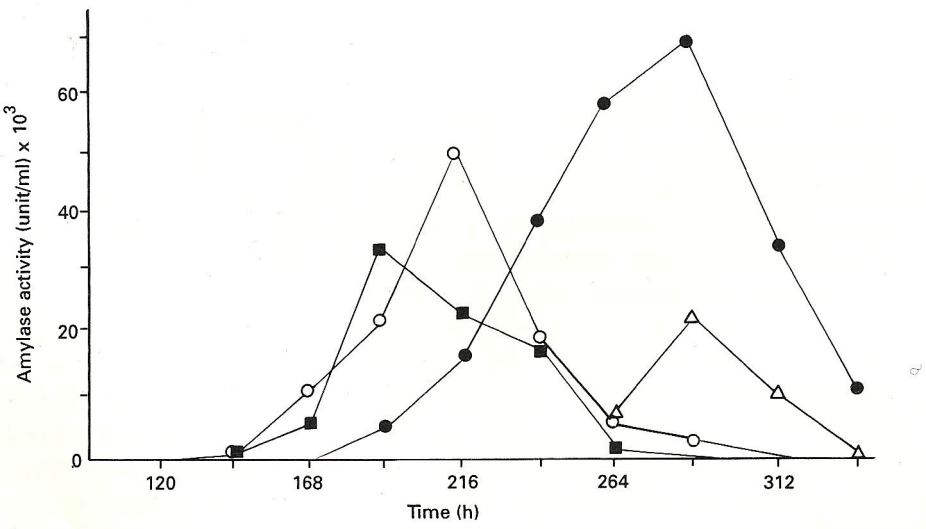

Figure 2 Effect of starch concentration on amylase production by *B. stearothermophilus* in corn steep liquor-soya bean meal medium. ■, 0.5% (w/v) of soluble starch; ○, 0.1% (w/v) of soluble starch; ●, 2.0% (w/v) of soluble starch; and △, 5.0% (w/v) of soluble starch.

The protein and amino acid contents of the liquor increased during the steeping period. The nutritive value of the protein released depended on the physiological availability of its constituent amino acids to the micro-organism. Other factors which may affect the nutritive value of protein in the liquor include indigestible carbohydrates, the relative proportion of the different amino acids, and the presence of anti-nutritional substances (Khan and Ghafoor, 1978). Foda *et al.* (1973) reported that at least fifteen amino acids beside the peptides and proteins were precipitated by pH adjustments during steeping which increased the amino acid content.

During the trial fermentation, the concentration of starch in the medium decreased as amylase concentration increased (Table 2). Enzyme production started at 96 h and reached a peak at 240 h. Thereafter, the amount of enzyme produced decreased sharply. The growth of the organism reached a peak at 144 h and started steadily declining. High enzyme production commenced during the peak of growth and continued in the stationary and death phases of the organism. Coleman and Elliot (1962) reported that amylase production by *B. subtilis* did not start until the phase of most rapid cell growth.

The use of various concentrations of soya bean meal in the fermentation medium revealed that 0.5% (v/v) of soya bean supported the highest growth of the organism (Figure 1). Soya bean was reported to contain protein (40%), oil (20%), carbohydrate (35%) and ash (7%) by Leysen (1985). However, in the trial fermentation, 0.2% (v/v) of soya bean meal was employed and found to be adequate because a concentration greater than this led to the formation of flocs on the surface of the medium which interfered with the oxygen supply and aesthetic quality of the broth.

As shown in Figure 2, the amylase activity became clearly evident after 120 h. The highest production of amylase occurred in the broth with 2% (w/v) starch. This concentration was feasible for use in the commercial production of amylase by *B. stearothermophilus* since corn steep liquor contains extra soluble starch which would add to the starch content of the fermentation broth. A further increase in starch concentration beyond 5% (w/v) led to substrate inhibition of the enzyme.

Corn steep liquor is at present a waste by-product of several corn starch industries in Nigeria. Elsewhere in the world, corn steep liquor is used in the production of inositol, phytin, antibiotics and several other industrial fermentation products (Foda *et al.*, 1973). Soya bean is used in Nigeria for the production of livestock feeds, milk and oil.

Utilization of cheap agricultural products such as soya bean and corn steep liquor would contribute greatly to the economical production of amylase for local industrial use.

References

AKINRELE L. A. 1970. Fermentation studies on maize during the preparation of a traditional African starch-cake food. *J. Sci. Food Agric.* **21** 619–25.

AMUND O. O. and Ogunsina O. A. 1987. Extracellular amylase production by cassava fermenting bacteria. *J. indust. Microbiol.* **2** 123–7.

COLEMAN B. and Elliot W. H. 1962. Extracellular ribonuclease formation in *Bacillus subtilis* and its stimulation by actinomycin D. *Biochem. J.* **95** 699–706.

DRUMM T. D., Gray J. I. and Hosfield G. C. 1990. Variability in the major lipid components of four market classes of dry edible beans. *J. Sci. Food Agric.* **50** 485–97.

FODA M. S., Salem S. A., Hegazi S. M. and Badr-Eldin S. M. 1973. Biochemical studies for utilization of corn-steep liquor in production of proteins. *J. Sci. Food Agric.* **24** 17–22.

IKENEBOMEH M. J., Kiok R. and Ingram J. M. 1986. Processing and fermentation of the African locust bean (*Parkia filicoidea* Welw.) to produce dawadawa. *J. Sci. Food Agric.* **37** 272–82.

KEAY I. and Wildi B. S. 1970. Proteinases of the genus *Bacillus.* I. Neutral proteinases. *Biotechnol. Bioeng.* **12** 179–212.

KHAN M. A. and Ghafoor A. 1978. The effect of soaking, germination and cooking on the protein quality of mash beans (*Phaseolus mungo*). *J. Sci. Food Agric.* **29** 461–4.

LEYSEN R. I. 1985. Soybean and soybean products. Food and Agricultural Organisation Symposium. pp 21–7. Nairobi, Kenya.

MURATA T., Akazawa T. and Fukuchi S. 1968. Enzymic mechanism of starch breakdown in germinating rice seeds. 1. An analytical study. *Plant Physiol.* **43** 1899–905.

OKAFOR N. 1987. *Industrial Microbiology.* 413 pp. University of Ife Press Ltd, Nigeria.

PLAHAR W. A. and Leung H. K. 1982. Effect of moisture content on the development of carboxylic acids in traditional maize dough fermentation. *J. Sci. Food Agric.* **33** 555–8.

SOMOGYI M. 1945. A new reagent for the determination of sugars. *J. biol. Chem.* **160** 61–8.

STANBURY P. F. and Whitaker A. 1984. *Principles of fermentation technology.* 255 pp. Pergamon Press, New York.

YEMN E. W. and Willis A. J. 1964. Estimation of carbohydrates by anthrone method. *Biochem. J.* **57** 508–14.

Accepted 5 August 1997