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Full Length Research Paper

Nitrogen supplements effect on amylase production by *Aspergillus niger* using cassava whey medium

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The production of amylase by *Aspergillus niger* on three cassava whey media in liquid shake culture was compared. The supplemented cassava whey (SCW) medium exhibited gave amylase activity of 495 U/ml. Biomass cropped was 1.63 g/l in the SCW medium. Yeast extract employed as a nitrogen supplement increased biomass yield of *A. niger* to 2.75 g/l with maximum amylase activity of 643 U/ml. Sodium nitrate ($NaNO_3$) as nitrogen supplement had the lowest biomass yield of 0.77 g/l and amylase activity of 206 U/ml. Thus yeast extract as nitrogen supplement of cassava whey medium supported maximum production of amylase and biomass of *A. niger.*

Key words: Amylase, cassava whey, *Aspergillus niger*, yeast extract.

INTRODUCTION

Amylase is a commercially important enzyme in the starch bioprocessing and brewing industries responsible for breakdown of starch or glycogen into simple sugar constituents (Akpan et al., 1999; Aiyer, 2005). Starch hydrolyzing enzymes such as amylase has received a great deal of attention because of their benefits. Tremendous research effort have been made on the applications of amylase for the conversion of starch to sugars (Hyun and Zeikus, 1985) and is currently most widely utilized in biotechnological applications ranging from food, fermentation, textile to paper industries (Lin et al., 1997; Pandey et al., 2000; Kurosawa et al., 2006). Amylases are widely distributed in plants and animals (Aiyer, 2005) and the enzyme from microbial sources are generally used to meet the expanding industrial demands (Pandey et al., 2000; Kurosawa et al., 2006). Among the microorganisms, many fungi had been found to be good sources of amylolytic enzymes. Studies on fungal amylase especially in the developing countries have concentrated mainly on *Rhizopus* sp. and *Aspergillus niger* probably because of the ubiquitous nature and non fastidious nutritional requirements of these organisms (Abe et al., 1988).

Wastes from the agricultural products during processing such as cassava whey can be used for bioconversion to produce protein enriched foods and other forms of value added products like amylase (Okolo et al., 1995; Ubalua, 2007). This paper provides an information on the use of cassava whey in amylase production by *A. niger* and effects of nitrogen supple-ments on the yield of biomass and amylase.

MATERIALS AND METHODS

Raw materials and cultures

Fresh cassava whey samples were obtained from a small scale cassava grating and processing plant site at Isihor, Benin City, Nigeria. Collection was into 4.5 litre plastic container previously cleansed and rinsed with 70% ethanol and distilled water, successively. The samples were allowed to sediment out for 6 h, solids were removed and supernatants used immediately.

Known strain of *A. niger* identified by Oshoma and Ikenebomeh (2005) was obtained from the culture collection of the Microbiology laboratory of the University of Benin, Benin City. The culture was maintained on potato dextrose agar (PDA) at 28 ± 2°C.

Preparation of cassava whey and fermentation

Fermentation was carried out in an orbital shaker at 120 rpm using three trial media. The first medium, supplemented cassava whey (SCW) medium had the following composition per litre $MgSO_4$ (0.5 g), KH_2PO_4 (1.0 g), $CaCl_2$ (0.5 g), cassava whey (292 ml) and made

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Table 1. Changes in pH values of supplemented and unsupplimented cassava whey medium for 5 days fermentation period using *Aspergillus niger*.

Medium	pH					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
SCW	4.50	4.50	4.48	4.42	4.35	4.30
UCW	4.50	4.50	4.48	4.46	4.46	4.40
UICW	4.50	4.50	4.50	4.49	4.49	4.48

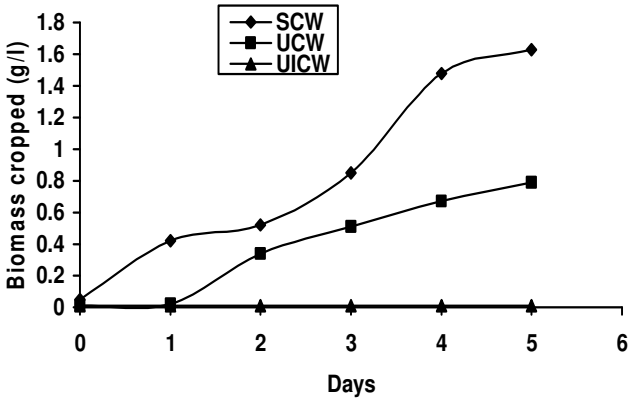

Figure 1. Total *Aspergillus niger* biomass cropped in supplemented and unsupplemeted cassava whey media for 5 days fermentation. SCW, Supplemented cassava whey medium with the following composition per litre $MgSO_4$ (0.5 g), KH_2PO_4 (1.0 g), $CaCl_2$ (0.5 g), cassava whey (292 ml) and made up to 1 litre with distilled water; UCW, unsupplemented cassava whey (UCW) medium had cassava whey (292 ml) made up to 1 litre with distilled water; UICW, uninoculated cassava whey medium with the same composition as UCW medium but was not inoculated.

up to 1 litre with distilled water. The second medium unsupplemented cassava whey (UCW) medium had cassava whey (292 ml) made up to 1 litre with distilled water. The third medium uninoculated cassava whey (UICW) medium had the same composition with the UCW medium but was not inoculated.

The effects of various nitrogen supplements and amylase activity was studied. SCW medium was used with each of the following nitrogen sources; yeast extract (2.0 g), $NaNO_3$ (4.5 g) and $(NH_4)_2SO_4$(3.32 g).

In all the media, initial pH was adjusted to 4.5 using 1 N HCl and/or 1 N NaOH. Each medium (98 ml) was transferred into 250 ml Erlenmeyer flask and sterilized at 121°C for 15 min. Inoculum size of 2.0 ml from homogenate of *A. niger* culture was aseptically transferred into each medium. Fermentation was at a temperature of 28 ± 2°C on an orbital shaker at a speed of 120 rpm followed by determination of amylase activity and other parameters at 24 h interval for 5 days.

Analytical methods

Amylase activity was assayed as described by Ramakirshna et al. (1982) using a reaction mixture comprising of 1 ml of crude enzyme, 1 ml of 1% starch solution and 0.1 ml of citrate buffer solution (pH 4.5). Incubation was at 60°C for 1 h and the reaction was terminated by immersing the reaction tube in boiling water (100°C) for 2 min. The reducing sugars liberated were estimated by the DNS methods (Miller, 1959). 1 unit of amylase activity (U) was defined as the amount of enzyme that liberated 1.0 µmole of D-glucose from starch in 1.0 µl reaction mixture under the assay conditions.

Dry mycelia biomass of *A. niger* was determined in the growth medium after each fermentation period. The mycelia were pasteurized at 65°C for 30 min in a water bath, removed from the flasks, placed on a dried and preweighed Whatman No 1 filter paper and washed twice with 50 ml of sterile distilled water. The biomass on the filter paper was dried at 90°C in a Genlab hot air oven YIA 110 model England until constant weight was obtained.

The initial and final pH values of fermentation media were determined using pH meter 3305 supplied by Jenway, England.

Titratable acidity was determined by transferring 10 ml of the filtrate into 250 ml Erlenmeyer flask and titrated against 0.1 N NaOH using phenolphthalein as indicator.

RESULTS AND DISCUSSION

A. niger was cultured in three liquid cassava whey media; supplemented cassava whey (SCW) medium, unsupplemented cassava whey (UCW) medium and uninoculated cassava whey (UICW) medium at 28 ± 2°C under shaking conditions. pH values (Table 1) were found to declined from 4.50 to 4.3, 4.40 and 4.48 for SCW medium, UCW medium and UICW medium, respectively. The titratable acidity had a corresponding increase of 13.60, 10.90 and 9.80 ml (0.01M NaOH) on day for SCW medium, UCW medium and UICW medium respectively (Table 2). Changes in reducing sugars values during the fermentation period are shown in figure 2. The values all declined on day 5 at 2.40, 2.50 and 5.70mg/ml for SCW medium, UCW medium and UICW medium respectively. The result of the dry biomass of *A. niger* peaked on day 5 at 1.63g/L, 0.79g/L and 0.01g/L for SCW medium, UCW medium and UICW medium respectively (Figure 1). The media resulted in varied amylase activity (Figure 3). The SCW medium had the highest peak of amylase activity of 495U/ml on day 3.

Investigations showed that of the 3 nitrogen sources (yeast extract, $(NH_4)_2SO_4$ and $NaNO_3$), yeast extract supported highest biomass of 2.75 g/l (Figure 4). Yeast extract also gave the best amylase activity of 643 U/ml (Figure 6) followed by $(NH_4)_2SO_4$ of 435 U/ml on day 3. From the 3 media tested in the study, supplemented cassava whey medium exhibited highest biomass cropped at 1.63 g/l on day 5 (Figure 1) and amylase activity of 495

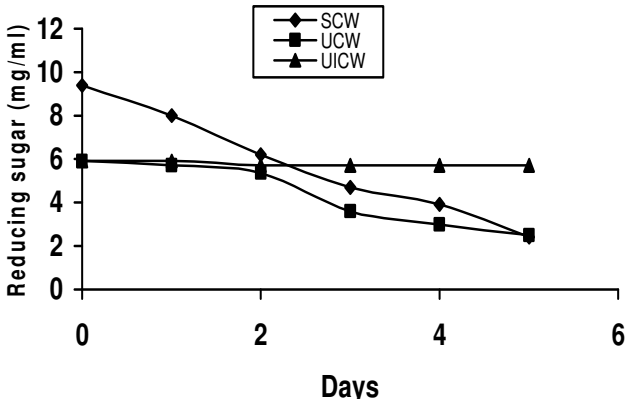

Figure 2. Changes in reducing sugar values of supplemented and unsupplemented cassava whey medium for 5 days fermentation period using Aspergillus niger. SCW, Supplemented cassava whey medium with the following composition per litre MgSO4 (0.5 g), KH2PO4 (1.0 g), CaCl2 (0.5 g), cassava whey (292 ml) and made up to 1 litre with distilled water; UCW, unsupplemented cassava whey (UCW) medium had cassava whey (292 ml) made up to 1 litre with distilled water; UICW, uninoculated cassava whey medium with the same compo-sition as UCW medium but was not inoculated

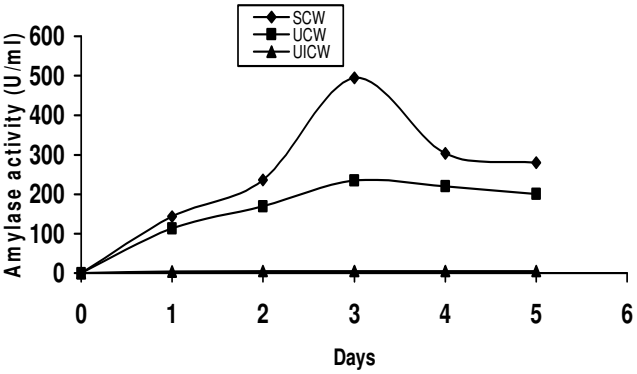

Figure 3. Total amylase production in supplement and unsupple-mented cassava whey medium with *Aspergillus niger*. SCW, Supplemented cassava whey medium with the following composi-tion per litre $MgSO_4$ (0.5 g), KH_2PO_4 (1.0 g), $CaCl_2$ (0.5 g), cassava whey (292 ml) and made up to 1 litre with distilled water; UCW, unsupplemented cassava whey (UCW) medium had cassava whey (292 ml) made up to 1 litre with distilled water; UICW, uninoculated cassava whey medium with the same composition as UCW medium but was not inoculated.

U/ml on day 3 (Figure 3) indicating that the cassava whey starch is a possible inducer of amylase production by *A. niger*. This possibility has earlier been reported upon by Akpan et al. (1999). Thus, it is clearly evident from the results of the present study that SCW medium appeared to support better growth and production of amylase by *A. niger*. Similar observation was made by Narasimha et al. (2006). Maximum efficiency of *A. niger* amylase was noted at 72 h by Omemu et al. (2005).

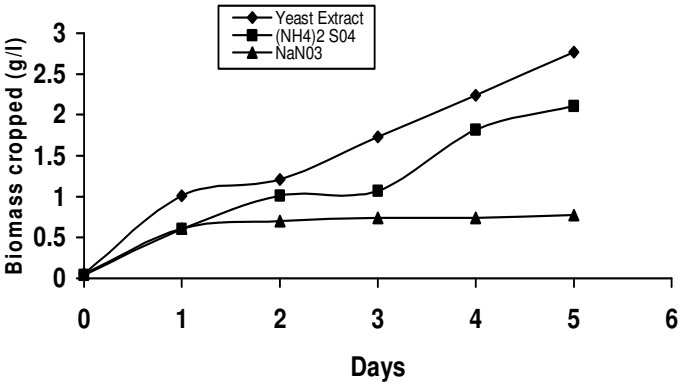

Figure 4. Effect of different nitrogen source in cassava whey medium on the dry biomass cropped of *Aspergillus niger* for 5 days fermentation period.

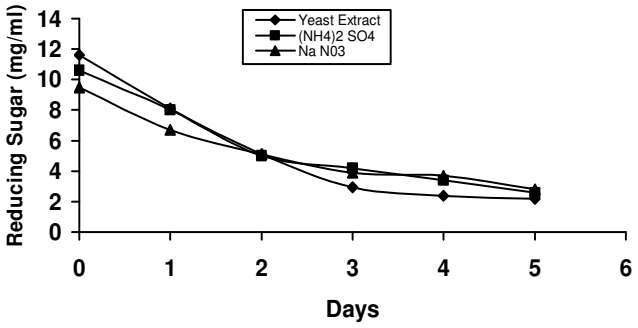

Figure 5. Effect of different nitrogen sources on reducing sugar in cassava whey medium for 5 days fermentation using *Aspergillus niger*.

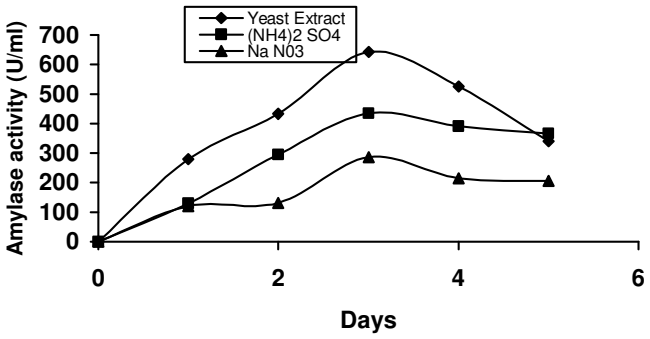

Figure 6. The effect of different nitrogen sources on *Aspergillus niger* amylase production.

Higher values of *A. niger* biomass were obtained with nitrogen supplementation and yeast extract gave the highest biomass yield (Figure 4). Since cassava is poor in protein content (Ugwu and Odo, 2008), any addition of nitrogen supplement would be expected to enhance bio-

Table 3. Changes in pH values of supplemented cassava whey medium (SCW) with different nitrogen source during 5 days fermentation at 28 ± 2°C by *Aspergillus niger*.

Medium	pH					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Yeast Extract	4.50	4.40	4.30	4.20	4.08	3.91
$(NH_4)_2SO_4$	4.50	4.44	4.35	4.26	4.08	4.09
$NaNO_3$	4.50	4.49	4.42	4.37	4.30	4.27

Table 4. Changes in titratable acidity [ml(0.1N NaOH)] values of supplemented cassava whey medium (SCW) with different nitrogen sources during 5 days fermentation at 28 ± 2°C by *Aspergillus niger*.

Medium	Titratable acidity ml (0.01 N NaOH)					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Yeast Extract	9.20	13.20	15.00	16.60	19.00	22.30
$(NH_4)_2SO_4$	9.30	12.60	13.40	15.40	17.40	20.40
$NaNO_3$	9.30	9.50	10.40	11.20	13.00	14.19

mass production. Yeast extract gave the highest biomass yield of 2.75 g/l (Figure 4), but according to Ikenebomeh and Chikwendu (1997), the preferred nitrogen source for *A. niger* biomass production was ammonium sulphate. Cassava is rich in starch hence the high values of the reducing sugars on day 0 of 9.40 mg/ml, 5.90mg/ml and 5.90mg/ml for SCW medium, UCW medium and UICW medium respectively (Figure 2), and 11.60, 10.60 and 9.50 mg/ml for Yeast Extract, $(NH_4)_2SO_4$ and $NaNO_3$ supplemented medium respectively (Figure 5). Consequent metabolism of *A. niger* resulted in the reduction of the sugars values. The utilization of sugars in the media by *A. niger* increased the production of amylase (Omonigho and Ikenebomeh, 2000).

Maximum amylase activity of 643 U/ml on day 3 (Figure 6) was achieved when yeast extract was the nitrogen source supplement. $(NH_4)_2SO_4$ gave higher amylase activity than $NaNO_3$. Hamilton et al. (1999) and Hayashida et al. (1988) reported that organic nitrogen sources are preferred for amylase production. They observed maximum amylase was produced when supported by yeast extract. Akpan et al. (1996) observed that increase in the organic nitrogen content enhanced amylase production to 411 U/ml by *Rhizopus* sp. which was far better than the inorganic nitrogen supplement.

Physical parameters such as pH of the growth medium play important roles by inducing morphological changes in microbes and enzyme secretion. The pH change observed during the growth of microbes also affects product stability in the medium (Gupta et al., 2003). The final pH values when yeast extract and $(NH_4)_2SO_4$ supplementations were used (Table 3) were 3.91 and 4.09, respectively. This agreed with Ikenebomeh and Chikwendu (1997) observation that nitrogen supplements increased acid production in the medium and this might eliminate the need for pH control equipment. Alva et al. (2007) reported that maximum amylase was produced at a pH of 5.8.

In conclusion, cassava whey medium with a relatively high starch concentration and simple nitrogen supplements (organic) can be successfully employed as a medium for the production of amylase using *A. niger*. Cassava whey is an agricultural waste, readily available and cheap in the tropics. *A. niger* could grow and produce the amylase at 28 ± 2°C, a common temperature in the tropics.

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