PRODUCTION OF *ASPERGILLUS NIGER* BIOMASS FROM AQUEOUS EXTRACT OF BREWER'S SPENT GRAIN

Aregbesola, O. A. and Omafuvbe, B. O. *
Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.
* Corresponding author: E-mail: bomafuv@oauife.edu.ng ; bomafuvbe@yahoo.co.uk
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Suitability of brewer`s spent grain (BSG) liquor for *Aspergillus niger* biomass (single cell protein) production was investigated. *Aspergillus niger* was grown in unsupplemented and supplemented (glucose or nitrogen sources [$(NH_4)_2SO_4$, $NaNO_3$, NH_4Cl, NH_4NO_3 and KNO_3]) BSG liquor by submerge fermentation on rotary shaker (120 rpm) at 28°C for eight days. *A. niger* cell biomass was harvested daily, pasteurized, centrifuged and dried (80°C). Glucose supplemented medium gave the highest biomass (1.64g dry wt. /L) and protein content (0.86g/L) at the 4th day of fermentation. $(NH_4)_2SO_4$ was the best N_2 supplement with a biomass yield of 1.43g dry wt. /L and protein content of 0.81 g /L. This study demonstrated that the production of *Aspergillus niger* biomass is a way of utilizing BSG (a low cost readily available by-product of brewing) to ensure a sustainable reuse of its bio-resources. It also established that *Aspergillus niger* biomass production can be enhanced by supplementation of BSG liquor with glucose or $(NH_4)_2SO_4$ with a maximum fermentation period of four days.

Key words: Biomass, Brewer`s spent grain, *Aspergillus niger*, Protein, Fermentation.

INTRODUCTION

Brewer`s spent grains (BSG) is the solid residue remaining after mashing and lautering, consisting primarily of grain husks and other residual compounds not converted to fermentable sugars by the mashing process. Brewer's spent grain (BSG) is the most abundant brewing by-product, corresponding to about 85% of total by-products generated (Xiros *et al*., 2008). Brewer's spent grain is available throughout the year with its main application limited to animal feeding. The chemical composition of BSG has been reported to vary due to the grain type or variety, time of harvest of the grain, malting and mashing conditions, and the quality and type of adjuncts added in the brewing process (Santos *et al.,* 2003). In brewing, the spent malted barley grains are approximately 80% cell wall material, with the remainder being mainly protein (Jay *et al*., 2008) and consist of about 16.8–21.9% cellulose, 28.4–29.6% hemicellulose and 21.7–23.0% acid insoluble lignin (Carvalheiro *et al*., 2004a, b ; Mussatto & Roberto, 2006). BSG has been used as an additive in cereal-based foodstuffs, energy production via direct combustion or anaerobic digestion to biogas, adsorbent for metals and dyes and for cultivation of mushrooms (Mussatto *et al.,* 2006). Other uses of BSG include: brick components, charcoal, paper manufacture, as growth medium for microorganism and enzyme production and feedstock production (Aliyu & Bala, 2011; Malomo *et al*., 2013). Attempts have been made to use BSG in biotechnology processes such as in single cell protein (SCP) production. Single cell protein is a microbial cell grown and harvested for animals or human food, due to its high protein content. *Aspergillus* sp. has been widely used for single cell protein production (Ravinder *et al*., 2003). The motive behind single cell protein production lies partly in the need for providing necessary food sources to solve the challenge generated by the rapidly increasing world population and partly in livestock feeding (Oscar *et al*., 2010). In particular, protein supply poses a problem since essential amino acids cannot be replaced. One possible solution to this problem is SCP production. While the intensive production of protein from animal sources requires large expanse of land and plants such as grasses for grazing or for production of animal food and feeds, single cell protein production requires limited land area.

In this study the suitability of BSG (a cheap and readily available brewing by-product) which is grossly underutilized in a developing country like Nigeria for the production of *Aspergillus niger* biomass (single cell protein) was investigated. In addition, the effect of nitrogen sources on biomass yield and protein content was studied for

possible optimization of the use of BSG.

MATERIALS AND METHODS

Source and Storage of BSG

Wet BSG were obtained from the International Breweries, Ilesha, Osun State, Nigeria. The wet BSG were dried in hot air oven at 60°C, milled and sieved (50 mesh size sieve) to remove the chaff and then stored in sealed containers until required.

Preparation of the Fermentation Medium

BSG liquor was prepared by steeping sixty grams of powdered BSG in 1 L of sterile distilled water for 24 h at 30°C, centrifuged (Centrifuge 80-2B) at 5000rpm for 30 min and the supernatant collected. The BSG liquor was used without supplementation (BSG) and supplementation with 2.0g glucose/L BSG (GBSG) or minerals {2.0g $(NH_4)_2SO_4$, 1.0g KH_2PO_4, 0.5g $MgSO_4.7H_2O$, and 0.1g $ZnSO_4.7H_2O$} made up to one litre with the BSG extract (MBSG). The effect of various nitrogen supplements on biomass production was tested on MBSG medium by substituting $(NH_4)_2SO_4$ (2.0 g/L) with each of $NaNO_3$ (2.6 g/L); NH_4Cl (1.6 g/L); NH_4NO_3 (1.2 g/L) and KNO_3 (3.0 g/L) to supply 0.42 g/L in the MBSG medium as previously described (Oshoma & Ikenebomeh, 2005).The supplemented and unsupplemented fermentation media were adjusted to pH 3.5 and autoclaved at 121°C for 15 min.

Isolation of *Aspergillus niger*

Aspergillus niger was isolated from bread left at room temperature for 2 weeks to undergo spoilage. The *A. niger* isolate was identified based on cultural characteristics and microscopy following standard methods (Barnett & Hunter, 1972; Rohde & Hartman, 1980) and maintained on potato dextrose agar (PDA) slant and stored at 4°C.

Preparation of Inoculum

A. niger was subcultured on PDA slant, incubated at 30°C for 7 days and the spores were harvested with sterile distilled water and standardized (10^6 spores/mL) as previously described (Raimbault, 1998).

Fermentation Process

The submerged fermentation method was employed. Briefly, sterile 100mL portion of each combination of the BSG liquor (supplemented and unsupplemented) in 250mL conical flasks were inoculated with 2.0 mL of *A. niger* inoculum (10^6 spores/mL) and left to ferment at 28± 2°C on a rotary shaker at 120 rpm for 8 days. Fermenting samples were collected at specified time intervals for analysis.

Biomass and Protein Content Determination

Fermenting sample of each BSG liquor combination was collected at 24 h interval, pasteurized in a water bath (Grant Instrument Type J-B2) at 95°C for 30 minutes, centrifuged (Centrifuge 80-2B) at 5000rpm for 30 min and the residue collected and dried at 70°C in a hot air oven (Astel Hearson Oven, England) to constant weight for biomass yield determination on dry wt. basis. The resulting dried cell biomass was stored in air tight containers until needed for analysis. The protein content of the dried cell biomass was determined following the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Statistical Analysis

Data obtained in this study were subjected to analysis of variance (ANOVA) and the Duncan`s multiple range test using the statistical package (SPSS 17).

RESULTS

The submerged fermentation of the BSG by *A. niger* showed that supplemented medium (GBSG and MBSG) gave a higher biomass yield of 1.64 g and 1.43 g (dry wt. /L) respectively, which was attained on the fourth day of fermentation while the unsupplemented liquor (BSG) gave the least biomass yield of 0.78 g dry weight/L on the eight day as shown in Figure 1. Glucose supplemented (GBSG) medium gave the highest biomass protein content of 0.86 g/L while BSG liquor gave the least biomass protein content of 0.606 g/L (Figure 2). Of the nitrogen supplements investigated, $(NH_4)_2SO_4$ gave the highest biomass yield of 1.43 g dry wt./L and protein content of 0.81 g/L while $NaNO_3$ had the least biomass yield of 0.84 g dry wt./L and protein content of 0.56

g/L (Figure 3 and 4). In general, the cell biomass of *A. niger* in submerged fermentation reached its peak by the fourth day of fermentation for the supplemented BSG liquor. Statistical analysis of the data reflected a significant difference in the dry cell biomass yield of *A. niger* in both supplemented and unsupplemented medium ($P < 0.05$).

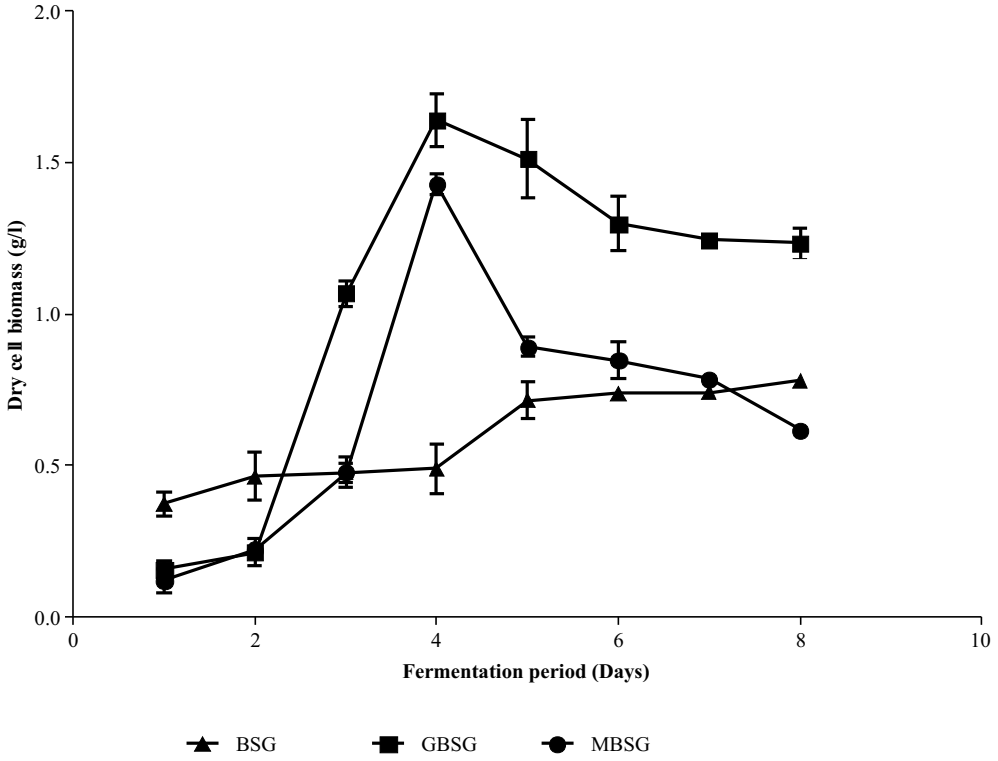

Fig.1: *Aspergillus niger* dry cell biomass yeild with fermentation period of brewer`s spent grain liqour. MBSG {brewer's spent grain liquor supplemented with 2.0g $(NH_4)_2SO_4$; 1.0g $KH_2PO_4$; 0.5g $MgSO_4.7H_2O$ and 0.1g $ZnSO_4.7H_2O$}; GBSG (brewer's spent grain liquor supplemented with glucose) and BSG (unsuplemented brewer`s spent grain liquor).

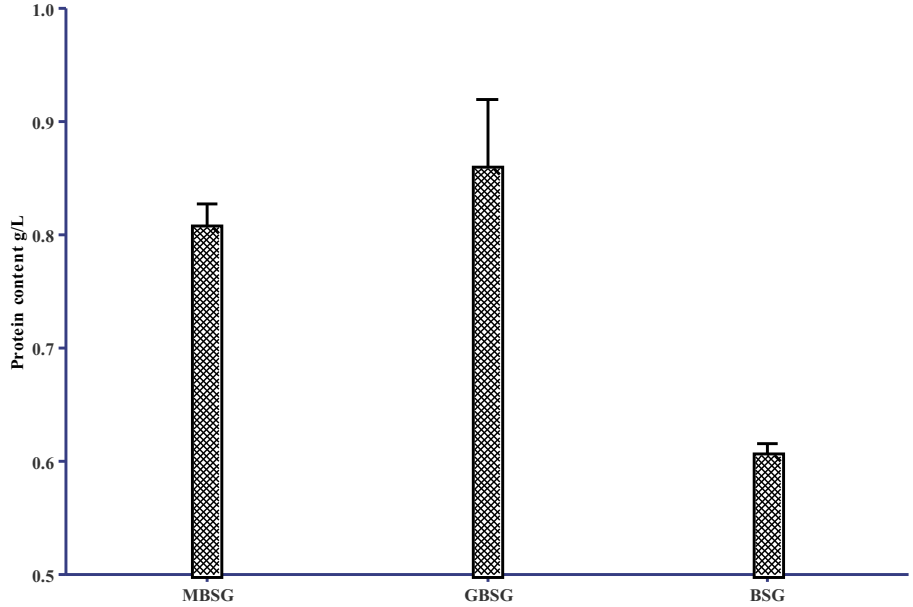

Fig. 2: Protein content (g/L) of *A. niger* biomass yield after 4 days of submerged fermentation. MBSG {brewer's spent grain liquor supplemented with 2.0g $(NH_4)_2SO_4$; 1.0g KH_2PO_4; 0.5g $MgSO_4.7H_2O$ and 0.1g $ZnSO_4.7H_2O$}; GBSG (brewer's spent grain liquor supplemented with glucose) and BSG (unsuplemented brewer`s spent grain liquor).

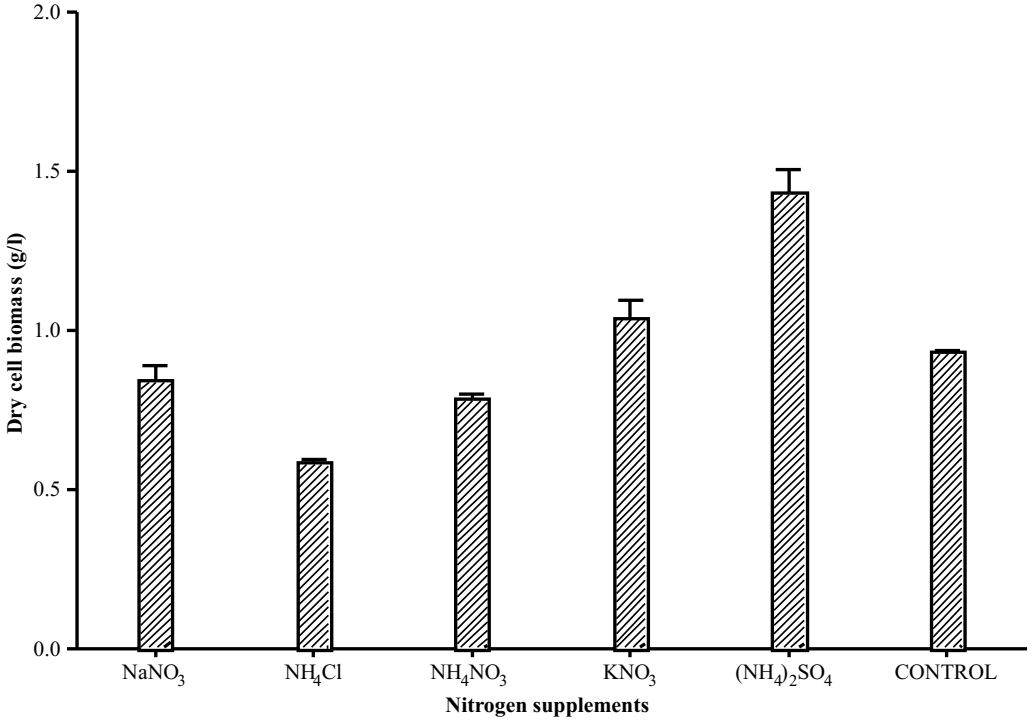

Fig. 3: Effect of various nitrogen supplements in MBSG on *A. niger* biomass yield after 4 days of submerged fermentation

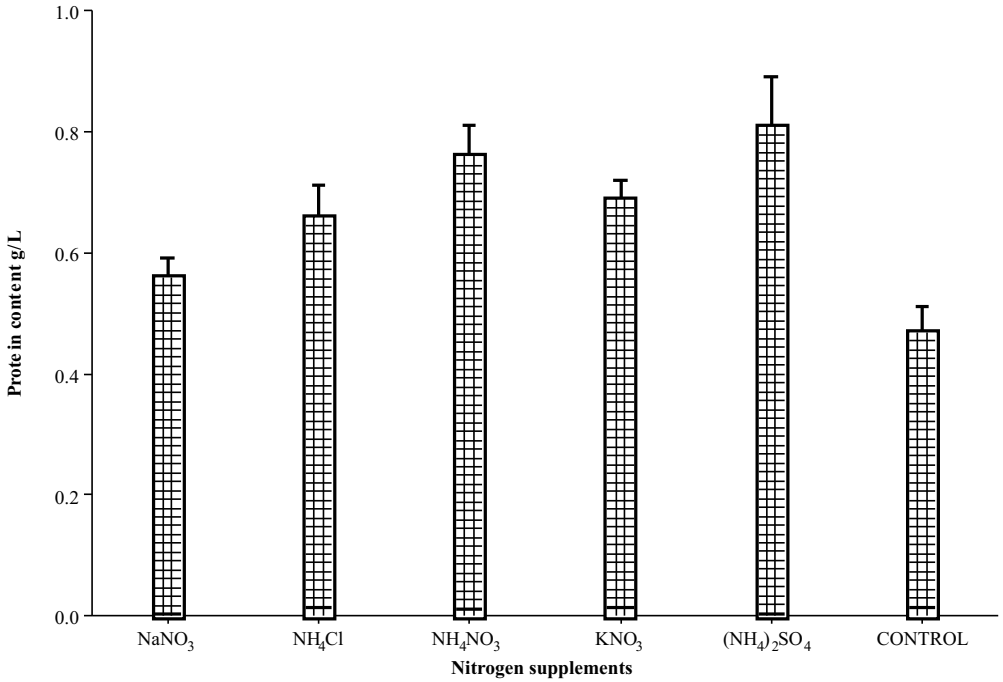

Fig. 4: Effect of different nitrogen supplements in MBSG on protein content of *A. niger* biomass yield after 4 days of submerged fermentation.

DISCUSSION

Supplementation of BSG liquor with glucose or mineral improved the biomass yields and protein content of *A. niger*. The total protein yield obtained in this study was higher than values reported for rice bran medium (Oshoma & Ikenebomeh, 2005). The result obtained shows the importance of supplementation of brewer`s spent grain extract for the production of A. *niger* (SCP) biomass. The low biomass yields obtained from the unsupplemented (BSG) medium could be as a result of limited concentration of nutrients most especially nitrogen which is required for microbial growth and biomass production. Availability of nitrogen supplements improved the biomass yield with $(NH_4)_2 SO_4$ giving the best yield, while $NaNO_3$ gave the least yield. This is in agreement with previous reports obtained with other agricultural and industrial waste substrates (Anupama & Ravindra, 2001; Oshoma & Ikenebomeh, 2005). The contributing factor for the low biomass yield of $NaNO_3$ has earlier been reported (Anupama & Ravindra, 2001), to be the reactivity of sodium ion. Sodium is higher than all the metals in the electrochemical series solution such as Zinc, Ferrous, Magnesium and Hydrogen ions in the activity series of metals. Hence, it displaces all these metals from their salts. The nitrate ions released from $NaNO_3$ into the medium may have reacted with the free cations of the mineral solution and formed nitrates. As a result of this, free nitrogen and also minerals solution controlling the pH was not available to support much growth of *A. niger*.

Maximum biomass yield with the supplemented medium was obtained after 4 days of fermentation followed by a decrease. The decrease in biomass production after the 4th day of fermentation may be attributed to nutrients depletion in the growth medium. This is similar to the report of Muhammad *et al.* (2010) on *Candida utilis*, but contrary to those of Lubna *et al.* (2004) and Khan *et al.* (1992), who reported maximum cell biomass of *A. niger* after 120 h and *Penicillium javanicum* after 144 h of fermentation respectively. The difference may be due to the inoculum and the medium type and composition. Analysis of variance (ANOVA) and Duncan's Multiple Range test on the dry cell biomass obtained with the incorporation of different nitrogen sources in brewer's spent grain medium showed a significant difference ($P < 0.05$) in the nitrogen supplements in response to the biomass yield.

In conclusion, this study has demonstrated a sustainable reuse of the bio-resources of BSG in the production of *A. niger* biomass. *Aspergillus niger* biomass production can be enhanced by supplementation of BSG liquor with glucose or $(NH_4)_2SO_4$ with a maximum fermentation period of four days.

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