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Construction of a Simple Multipurpose Airlift Bioreactor and its Application for Yeast Propagation and Amylase Production

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Abstract

Nigeria and other developing countries have abundant biomass resources that can be converted to many value-added products. However, these biomass resources are usually exported un-processed at very low prices because of lack of appropriate technology for processing. One of such limitations is lack of simple bioreactors for large scale cultivation of cells. The conventional bioreactors are usually very complex, expensive and require constant power supply for mixing and agitation. They are therefore not suitable for developing countries where electricity supply is very un-steady. The aim of the present research is to develop a simple airlift bioreactor which can be operated even without electricity supply. The bioreactor was constructed of acrylic panels and demarcated into a riser and a down comer chambers. The total volume of the bioreactor was 3.9 L with a working volume of 3.0 L. Mixing was achieved by aeration using mechanically operated gasoline-powered air compressor. Mixing efficiency and thus dissolved oxygen concentrations can be controlled by adjusting the aeration rate. The efficacy of the bioreactor was demonstrated by cultivation of facultative aerobic microorganism (Saccharomyces cerevisae) and aerobic microorganism (Aspergillus awamori). At both low sugar concentration (50 g/L) and high sugar concentration (100 g/L), the growth rates and thus the final concentrations of Saccharomyces cerevisae biomass were higher in the bioreactor than in Erlenmeyer flasks. Also in comparison with flask cultures, cultivation of Aspergillus awamori in the bioreactor resulted in higher gluco-amylase and α-amylase activities.

Key Words: Bioreactor, Aerobic microorganism, Acrylic panels, *Aspergillus awamori*

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Introduction

Nigeria and most other developing countries have abundant bio-resources that can be processed into value added products. Presently, these biomass resources such as agricultural produce are exported either un-processed or semi-processed at very cheap prices. They are processed in developed countries and exported back to the developing countries at very high prices. For example, Nigeria is the world larges producer of cassava (FAO 2004) but exports cassava chips which are very cheap, bulky and highly perishable. Local processing of cassava into such products as glucose syrups (by hydrolysis), or other fermentation products such as ethanol (Roble et al., 2003a; Zhang et al., 2003; Dai et al., 2006; Ogbonna and Okoli, 2009), amino acids, and organic acids (Roble et al., 2003b) will increase her export earning and create employment opportunity for the teaming un-employed youths. However, large scale bio-processes such as cell cultivation, fermentation, production of various metabolites, and enzymatic processes require bioreactors. Presently, various types of bioreactors have been developed and currently used for production of useful metabolites by various microbial, plant and animal cells. On the basis of mixing, bioreactors can be classified into mechanically agitated bioreactors and non-mechanically agitated bioreactors (Aiba et al, 1965). The mechanically agitated bioreactors are equipped with impellers and require constant power supply for rotation of the impellers. These types of bioreactors are more complex and thus not suitable for developing countries that do not have constant power supply. On the

other hand, the non-mechanically agitated bioreactors are mixed by aeration and the hydrodynamic stress is relatively low. They are very simple, cheap and thus are very suitable for developing countries (Okafor, 2007). The aim of this study was to construct a simple non-mechanically agitated (air lift) bioreactors which can be operated in rural areas without electricity supply. The effectiveness of the constructed bioreactor was demonstrated by cultivation of *Saccharomyces cerevisiae* for production of yeast inoculum and *Aspergillus awamori* for production of amylases.

Materials and Methods

Bioreactor design criteria: The major criteria considered in the design of the bioreactor include costs, simplicity, hydrodynamic stress, mass transfer efficiency, and the feasibility of operating in rural areas without electricity supply.

Description of the bioreactor: Schematic diagram of the simple airlift bioreactor is shown in Fig.1. It is constructed of acrylic panels. The total height is 31.5 cm, the length is 13.5 cm and the weight is 10.5 cm, giving a total volume of 3.9 L but a working volume of 3.0 L. It was split into a riser and a down comer compartments. The riser chamber is equipped with a ring sparger. The sparger was made of perforated rubber tubing with internal compression metal. The bioreactor is mixed and aerated by bubbling air from gasoline engine- powered air compressor through the sparger. The aeration rate can be controlled to achieve optimum mass transfer for either enzyme or yeast inoculum production.

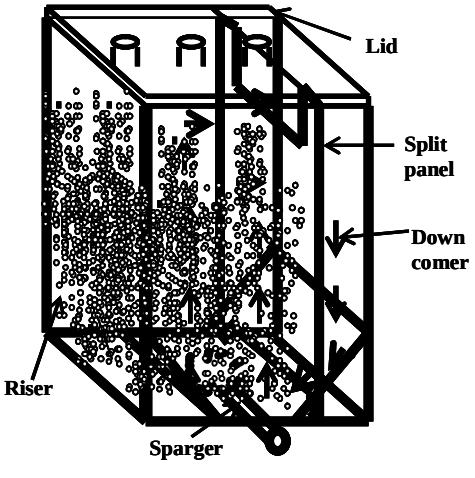

Fig.1. A multi-purpose airlift bioreactor for enzyme and yeast inoculum production.

Enzyme production by Aspergillus awamori in the airlift bioreactor: The composition of the medium used for enzyme production in the airlift bioreactor consisted of starch (soluble or cassava), 20g /L, polypeptone 5 g/L, yeast extract, 2 g/L; KH_2PO_4, 1 g/L; $CaCl_2.2H_2O$, 2 g/L; $MgSO_4.7H_2O$, 1 g/L and anti-foam agent, 0.1 mL. The medium (3.0 L) was autoclaved and transferred into the sterilized bioreactor. The bioreactor was sterilized by washing very well and filling with 0.1% sodium hypochlorite overnight, followed by rinsing with sterilized distilled water. The reactor containing medium was then inoculated with *Aspergillus awamori* spores (initial concentration = 6.6 x 10^6 spores/mL). Aeration was controlled at 0.2 vvm (volume of air per volume of culture broth per minute) using an air flow meter. The amylase activities were measured every 24 h for 96 hours. After 96h the liquid medium was centrifuged at 3000 rpm and the supernatant was decanted into sterile flasks and used as the crude liquid enzyme.

Production of yeast inoculum using the new bioreactor: The composition of the medium used for yeast cultivation in the airlift bioreactor include yeast extract, 2.5g/L; polypeptone, 2.5g/L and glucose, 20g/L. All the media components were dissolved in tap water in a 1000ml Erlenmeyer flasks and then autoclaved at 121^{o}C for 15 minutes. The autoclaved medium was transferred into sterilized bioreactor. Sterilization of the bioreactor was as described for cultivation of *A. awamori*. The aeration rate was 1.0 vvm. Two experiments were performed with initial glucose concentrations of 50 g/L and 100 g/L respectively. After the cultivation, aeration was stopped and the yeast cells sedimented at the bottom of the bioreactor. The cells were then harvested by decanting.

Analytical methods: The α-amylase activity in the crude enzyme was measured by the method developed by (Bailey and Olis, 1986). α-amylase activities were estimated by measuring the quantity of starch degraded in 1 minute. The α- amylase activity was defined as the amount of enzyme that degrades 1µg of starch in 1 minute under the experimental conditions. Gluco- amylase activity was estimated by measuring the reducing sugars released from soluble starch. One unit of glucoamylase was defined as the amount of enzyme that liberates 1µg of reducing sugar in 1 min. Ethanol concentration was determined by the boiling iodometric method (Adetuyibi, 1989).

Statistical analysis: All the experiments were done at least three times. The standard

error of the mean and confidence limits were calculated and the mean values were plotted with 95% confidence limits (Parker, 1980). Significance differences between treatments were tested by analysis of variance (ANOVA) and the means were separated by calculating the Least Significance Differences (LSD).

Results

Enzyme production by Aspergillus awamori in an airlift bioreactor: A comparison of α-amylase production in shake flask and the airlift bioreactor is shown in Figure 2. In both vessels, the α-amylase activity increased almost linearly with cultivation time. However, the α-amylase activity was higher in the bioreactor than in the flask throughout the cultivation period. Also in the case of gluco-amylse, the enzyme activity was significantly higher in the bioreactor than in the shake flask (Figure 3).

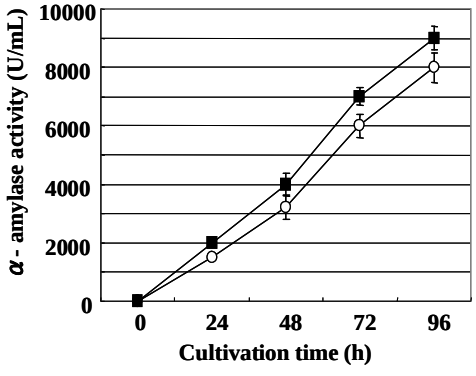

Figure 2. Comparison of α - amylase production in flask (○) and the constructed bioreactor (■).

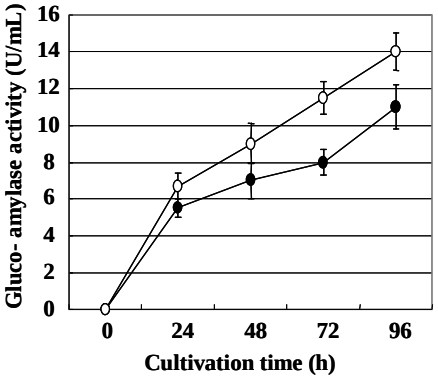

Figure 3. Comparison of gluco amylase production in flask (●) and the constructed bioreactor (○).

Production of yeast inoculum using the new bioreactor: A comparison of yeast inoculum production in flask and the bioreactor is shown in Figure 4. With the same initial sugar concentration of 50 g/L, yeast growth rate and the final biomass concentration were higher in the bioreactor than in the flask. Even when the initial sugar concentration was increased to 100 g/L (Figure 5), the bioreactor also resulted in higher yeast concentration. The working volume of the bioreactor was 3.0 L as against 200 mL in the flask. Thus with an initial glucose concentration of 100 g/L, the total amount of yeast inoculum produced in the bioreactor was 78 g as against 4 g produced in the flask.

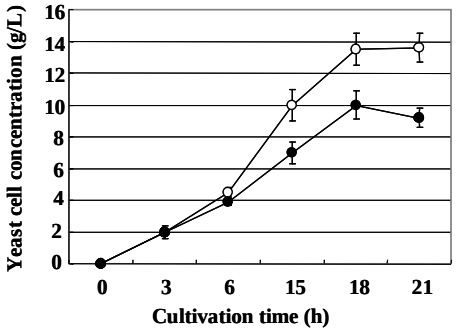

Figure 4. Comparison of yeast seed culture production in flask (●) and the constructed bioreactor (○) with an initial glucose concentration of 50 g/L

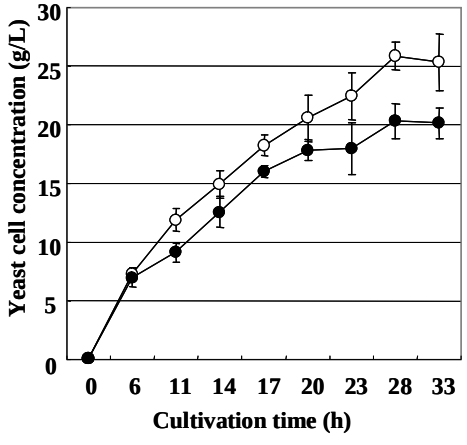

Figure 5. Comparison of yeast seed culture production in flask (●) and the constructed bioreactor (○) with an initial glucose concentration of 100 g/L

Discussion

Production of sufficient amount of crude liquid enzyme for large scale processing of starchy agricultural produce cannot be done in flasks. In this study a simple and cheap bioreactor that can be locally fabricated and operated even without electricity for production of large quantities of liquid enzyme and yeast seed culture has been developed. Enzyme production is an aerobic process that requires bioreactor with good mass transfer characteristics. Furthermore, efficient ethanol production and bakery industries require large quantities of active yeast cells. In a small scale, the yeast inoculum can be produced in

flask cultures. However, the amount of yeast cells required for large scale ethanol production cannot be produced in flasks. It is also known that aerobically propagated yeast cells store fatty acids and sterols which enable it tolerate anaerobic condition during ethanol fermentation (Jones and Ingledew, 1994). In view of these, a multi-purpose airlift bioreactor which can be used for production of both crude enzyme and yeast inoculum was developed. An advantage of the airlift bioreactor is that it is operated by air-compressor which can be operated without electricity. Shake flasks and mechanically agitated bioreactors require constant power supply and are not suitable for places without, or with unsteady power supply. Although different types of airlift bioreactors have been developed by various researchers, this study is the first report on the operation of an airlift bioreactor with manual air compressor, demonstrating that it can be efficiently operated in rural areas without electricity supply. As expected, the amylase activities and the inoculum yeast concentrations obtained in the airlift bioreactors were higher than those obtained in flask cultures. Thus, aside from making it possible to produce large amounts of the amylase enzymes and yeast inoculum, the specific activities of the products are also higher.

References

Adetuyibi, A. (1989). Introduction to Biomedical Research. University Press Ltd. Ibadan 27

Aiba, S., Humphery, A. E., and Millis, N. F. (1965). Biochemical Engineering. University of Tokyo Press, Tokyo Japan.

Bailey, J. E. and Olis, D. F.(1986). Biochemical engineering Fundamentals, 2nd Edition, McGrow –Hill 270-277.

Dai, D., Hu, Z., Pu, G. Li, H. and Wang C. (2006). Energy efficiency and potentials of cassava fuel ethanol in Guangxi region of China, *Energy Conversion and Management* 47, 1686-1699.

Food and Agriculture Organization of the United Nations (2004) www.fao.org

Jones, M. A. and Ingledew W.M. (1994) Fuel alcohol production: Appraisal of nitrogenous yeast foods for very high gravity wheat mash fermentation, *Process Biochemistry* 29, 483-488.

Ogbonna J.C. (2004). Fuel ethanol production from renewable biomass resources: Feed stocks and process strategies. In Pandey. A (Ed) Concise Encyclopedia of Bio-resources Technology. Haworth Press. New York.

Ogbonna, C. N. and Okoli, E. C. (2009). Evaluation of some cassava varieties in Nigeria for Bio-ethanol production. Bio-research. In press.

Okafor, N. (2007). Modern Industrial Microbiology and Biotechnology. Science Publishers, USA. Pp. 183-206.

Parker, P. E. (1980). Introductory Statistics for Biology. Second Edition Edward Arnold Publishers Ltd. London.

Roble N. D., Ogbonna J. C., Tanaka H. (2003a). A novel circulating loop bioreactor with cells immobilized in loofa (*Luffa cylindrica)* sponge for bioconversion of raw cassava starch to ethanol. Appl. Microbiol. Biotechnol.60, 671-678.

Roble N. D., Ogbonna J. C., Tanaka H. (2003b) L-Lactic acid production from raw cassava starch in a circulating loop bioreactor with cells immobilized in loofa (*Luffa cylindrica)* sponge. Biotechnol. Lett. 25, 1093-1098.