PRODUCTION OF SINGLE CELL PROTEIN USING AGRICULTURAL WASTES

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Abstract
Investigation was carried out in the Laboratory of the Department of Plant Science, Imo State University, Owerri on the production of single cell protein by yeast using agricultural wastes (Pineapple peels, saw dust, rice bran, brewer's waste). Result shows that moisture content in pineapple was 82 %, 46 % in saw dust, 43 % in rice bran and 50 % in brewer's waste and all were statistically significant. The reducing sugar was 9.9 % in pineapple, 3.8 % in saw dust, 3.6 % in rice bran and 4.2 % in brewer's waste. Pineapple recorded 11.5 % non reducing sugar, dust 5.2 %, rice bran 4.8 % while brewer's waste was 9.6 %. The crude protein in pineapple was 0.08 %, 0.40 % in saw dust, 0.32 % in rice bran, and 0.53 % in brewer's waste. The non reducing sugar and crude protein were not statistically significant among the four substrate (P=0.05). Pineapple waste recorded lowest fresh weight 0.03 g, saw dust 2.70 g, brewer's waste 2.60 g while rice bran was highest 2.80 g. Pineapple waste had 0.89 g dry weight, saw dust 0.83 g, brewer's waste 3.91 g and rice bran 0.98 g respectively. Investigation revealed that pineapple produced the highest quantity of single cell protein. Therefore, it should be preferred as substrate for single cell protein more than other substrates.

Keywords: Single Cell, Protein, Production, Agricultural wastes.

Introduction

Proteins are biochemical compounds consisting of one or more polypeptides typically folded into a globular or fibrous form facilitating biological functions. Like other biological macromolecule proteins such as polysaccharides and nucleic acid, proteins are essential part of organisms and participate in virtually every process within cells. Apart from the usefulness to humans, they are also necessary in animal diet. Protein is a major constituent of cell and it is important for proper growth and development of the human body. Its deficiency causes serious disorder such as stunted growth, lack of formation of cells, even exposure to diseases and other health problems.

Most of the increasing world population are facing a major problem in malnutrition due to rapid growth in their populations and decrease in sources of protein. Pressure is exerted on the food industries to produce enough animal food and also for human to meet up with the nutritional value of food requirement. This cancer has led to the exploitation of non conventional food sources as potential alternatives (Singh, 1998). Because of the intensity in the price for traditional protein ingredient for animal foods, there is great need to find new sources of protein that will not require agricultural land, costly and tedious means of production (Saquido *et al.*, 1983).

Due to increased world demand for cheap and affordable sources of food a as well as search for a non conventional way of solving the problems of the world protein shortage, single cell protein was introduced (Schlegel, 1999). In animal feeding and nutrition, single cell protein has application as fattening of calves, poultry, pigs and fish breeding. In food also, it is used as aroma carriers, vitamins, emulsifying agents and improved the nutritional value of blacked food.

There have been studies as well as efforts to improve the protein quantity and quality of finished food products by augmenting protein rich cheaper ingredient in food formation (Nasir and Butt, 2011). Although, animals are considered to be the best quality of protein, microbial protein also known as single cell protein (SCP) growth on agricultural wastes is one of the important optional proteins because of higher protein content and very short growth cycle of micro-organism, thereby leading to rapid biomass production (Bacha *et al*., 2011).

Agricultural wastes are basically the most useful substance for the production of single cell protein. In recent years, wastes such as pineapple peels, rice bran, saw dust, brewer's waste and other industrial wastes has been used as substrates for growing single cell protein (SCP) and its production. This is because proteins from other sources are costly and are not much affordable for the poor masses, but the use of agricultural wastes has been found to be very cheap in the production of high quality protein. They are also easy to obtain. Because of this big gap between the demands of protein rich food and its supply to the ever increasing world population, in other to bridge the gap, single cell protein is an innovative and alternative way of feeding the world with protein especially under developed countries like Nigeria that greatly depend on the importation of protein source from other countries. Hence the objectives of the study are to produce cell protein from agricultural wastes, to evaluate the quantity of the protein produced using bacteria, fungi and algae and to solve the problem of malnutrition through the production of single cell protein.

Single Cell Protein (S.C.P.) is generated from various agricultural waste and they are potentially valuable nutritional constituent which when processed could yield food, feed, fuel, chemicals and minerals (Anupama and Ravindera, 2000; Han, 1975). Since 2500BC, yeast has been used in bread and beverage production. In 1781, it was processed for preparing highly concentrated forms of yeast as Biomass that is very rich in protein (Adoki, 2008 ; Abou-Hamed, 1993).

Single Cell Protein (SCP) are seen as microbial organism which are allowed to grow on waste products especially agricultural wastes as well as agro based Industries and they produce large quantity of protein and store them in their cell bodies. Interest in microbial production is increased because micro-organisms can utilize the waste materials that cause pollution problems and or sanitary hazards. Organisms that act on agricultural wastes are considered to be useful subtracts for production of microbial protein, but they must meet up with the following demand:

It should be none toxic, abundant, none exotic, totally regenerable, cheap and able to support growth and multiplication of the organism resulting in high equality biomass (Maini and Sethi, 2000). With the present alarming danger, the world will soon be unable to feed its population because of inadequate and shortage of food supply (mainly protein rich food). Therefore, the development of novel food production independent of agricultural land use is thus becoming imperative (Anupama and Ravindera, 2000).

Some Agricultural residues when dumped in an open environment constitute health hazard due to pollution and support of the microorganisms such as *Actinomycetes*, fungi and bacteria (Barton, 1978). Recycling of microbial organism can be achieved naturally and artificially by micro-organisms. Aerobic organisms such as fungi, bacteria and some anaerobic organisms have shown to be able to degrade some constituent of these residues.

Fungi play a significant role in the degradation of cellulose under aerobic conditions. Schlegel (1999), further explains that microbial organisms are potential matter that are such in the three groups of vitamins and also in protein that contains essential amino acids and these constitute the potential enrichment for protein deficiency foods.

Protein are present in all living tissues as building block components of the body and it contain dietary components for the supply of nitrogen as well as sulphur and it may be used as human food supplement or animal feed protein (Khan *et al*., 1992).

Single Cell Protein (SCP) production has the potential for feeding the ever world increasing population at cheaper rates. Najafpur, (2007) suggested the potential of (SCP) to overcome shortage of food in the world. Many researchers in their various investigations have used inorganic supplement for the mycelium growth on waste materials. (Yabaya and Ado, 2008; Ojokoh and Uzoh, 2005; Enwefa, 1991; Moharib, 2003).

Anupama and Ravindera, (2000) observed a continued population growth and the diminishing effect of protein rich food especially in developing and third world countries, a situation that possess serious threat to food security. Due to yawning gap in demand and supply. They described this situation as leading to chronic malnutrition.

Single Cell Protein develops when microbes ferment waste materials including wood, straw cannery and food processing wastes residues from alcohol production, hydrocarbons, human and animal excreta. The problem of extracting single cell protein is the dilution and cost. They are found in very low concentration; usually engineers have developed ways to increase the concentration including coagulation and filtration, or the use of semi permeable membrane. Some amino contaminants can produce mycotoxins. Some bacteria single cell proteins have amino acid profiles different from animal protein yeast and fungal protein tends to be deficient. Microbial biomass has high nucleic acid content and levels must be limited in the diets of monogastric animals to 50g per day (Riviere, 1977).

Materials and Method:

The ripped yellowing pineapple waste was obtained from World Bank market and Ekeonunwa market all in Owerri, Imo State and rice bran was obtained from Abakiliki. The Brewers waste was collected from a bear production industry located at Awomama in Imo State while Saw dust was obtained from Ogbsisi, Timber market in Owerri, Imo State. Also the single cell used was yeast and it was obtained from Food Science and Technology laboratory, Imo State University.

The chemicals KH_2PO_4, $MgSO_4.7H_2O$, NaCl, $CaCl_2$, Peptone water, Urea, were obtained from Gadis Nigeria Limited Owerri, Imo State. These chemicals help to induce the nutrient media for the growth of single cell.

The quantity of all the four substrate used were obtained using a precision weighing balance and then they were used to determine the weight of the wet and dry biomass of the single cell.

The samples were dried using an electric ovum so as to remove their moisture contents and one litre of water obtained using a graduated beaker

Media preparation: The pineapple wastes were washed to remove dirt and other impurities. Four samples were later dried using electric ovum at a temperature of 50^oC for 24 hours to remove the moisture content. The samples were later heated to dryness and ground to a fine powder and then sieved to remove the impure ones.

Method of preparation: The four samples were sieved into fine powder other than the control. They were weighed in equal amount and introduced into a sterilized beaker as presented in (Table 1).

Table 1: Method of Preparation of Samples:

Chemicals	Pineapple wastes	Saw dust	Brewers wastes	Rice bran	Control
$(NH_2)SO_4$	5.0g	5.0g	5.0g	5.0g	Gluc, Yeast & 1 lit H_2O
KH_2PO_4	1.0g	1.0g	1.0g	1.0g	
$MgSO_4.7H_2O$	5.0g	5.0g	5.0g	5.0g	
NaCl	0.1g	0.1g	0.1g	0.1g	
CaCl	0.1g	0.1g	0.1g	0.1g	
Distl Water	1.0 lit	1.0 lit	1.0 lit	1.0 lit	
Peptone water	10.0 ml	10.0 ml	10.0 ml	10.0 ml	
Urea	3.0g	3.0g	3.0g	3.0g	

Fermentation: At the end of the preparation, the medium were distributed in a sterilized beaker. The yeast strains were inoculated in the media and incubated at 28^oC for seven (7) days to deduce the biomass increase. The yeast cells were separated by washing from the fermented broth and analyzed.

Data Analysis: Data was analyzed according to the method of SAS, (1996).

Results

Result of the investigation revealed that there was no biomass increase on the first and second day, but on the third day there was an increase that seems to be equal in all four samples. On the fourth day, there was more increase in the beaker containing pineapple wastes, followed by saw dust while rice bran and brewer's wastes seems to maintain the same level of biomass increase.

On the fifth and sixth day, there was greater increase in the pineapple wastes and there was no increase on the biomass at seventh day. Result showed that the moisture content of pineapple wastes was significantly high 82 %, followed by Brewers wastes 56 %, and then saw dust 46 % when rice bran 42 % was the lowest. (Table 2).

Pineapple wastes also produced the highest amount of reducing sugar 9.90 % followed by Brewers wastes 4.2 %, and then saw dust 3.80 %, when rice bran 3.60 % was lowest. The none reducing sugar of the pineapple wastes was highest 11.50 %, followed by brewers' wastes 9.60 %, then saw dust 5.20 % when rice bran 4.80 % was the lowest. The crude protein in pineapple wastes was found to be 0.80 % as the highest, followed by brewers' wastes 0.53 %, then saw dust 0.40 % when rice bran 0.32 % was the lowest (Table 2.)

Result showed that pineapple has had the highest wet biomass 3.03 gm, followed by rice bran 2.7 gm, and then saw dust 2.70 gm and brewers' wastes had the lowest 2.60 gm. On the dry biomass, rice bran recorded the highest 0.98 gm, followed by brewers wastes 0.91 gm then pineapple wastes 0.89 gm while saw dust had the lowest 0.83 gm (Table 3).

Investigation revealed that moisture content of pineapple was found to be 22.65 % followed by brewer's wastes 11.65 %, and then saw dust 6.45 % while rice bran 4.50 % was the lowest. Pineapple substrates produced the highest amount of reducing sugar 10.20 % followed by brewer's wastes 5.68 %, and then saw dust 3.80 % and rice bran had the lowest 3.25 %. It was observed that pineapple had the highest none-reducing sugar 300 % followed by brewer's wastes 288 % then saw dust 156 % and rice bran 126 % appearing as the lowest (Table 4).

Discussion

The media waste analysis showed that all the compositions of the agricultural wastes investigated upon were statistically significant on moisture content at 5% probability level. This is in accordance with the findings of Dhanasekaran *et al*. (2011), Bacha *et al*., (2011) as well as Ojokoh and Uzeh, (2005). The high moisture reducing and none reducing sugar and crude protein recorded in pineapple wastes makes it a better substrate for single cell protein production than other wastes products considered in the research. Also the high yield of biomass observed in pineapple further affirms its position as the best substrates for single cell protein production.

The none significant difference in the substrates on dry biomass weight shoed that any of them can be a good substrate. However, high wet biomass produced in pineapple medium was in agreement with the findings of Enwefa, (1991) who reported that the wet biomass produced in pineapple medium was higher than basal media and also in line with Bacha *et al*. (2011) who reported high weight biomass in potato waste than various agro industrial waste used in the production of single cell protein.

The high percentage crude protein recorded by pineapple in this study was in accordance with Martin *et al*. (1993) and Enwefa, (1991). The high level of difference observed in pineapple waste when compared with other substrates in all the parameters on wet biomass makes it more suitable for single cell protein production.

In conclusion, different agricultural waste such as pineapple waste, rice bran, brewer's waste and saw duct could be used in the production of single cell protein and pineapple waste is recommended as the best substrate as it produces the highest quantity of single cell protein followed by saw dust while rice bran was the lowest.

References:

Abou Hamed, S.A.A. (1993). Bioconversion of Wheat Straw by Yeast into Single Cell Protein. *Egypt. J. Microbial* 28(1): 1-9.

Adoki, A. (2008). Study of the factors affecting Yeast growth and Protein yield production from orange, plaitain and banana wastes. *African J. Biotech* 7(3): 290-295.

Anupama, A. and P. Ravindera, (2000). Value added food for single cell protein. *J. of Bioitechnology*. 7(3): 290-295.

Barton, G.J. (1978). Microbial bioconcentration of organic pollutants from agricultural residues. *Appl. Environ Microbial* 57(2): 563-567.

Bacha, U., Nasir, M. Khalique, A. Anjum, A.A., and Jabbar, M.A. (2011). Comparative assessment of various Agro Industrial wastes for *Saccharomyces cerevisiae* biomass production wastes for evaluation as single cell protein. *Journal of animal and plant science* :21 (4): 844-849.

Dhanasekaran, D., Lawanya, S., Saha, S., Thajuddin, N. and Panneerselvam, A. (2011). Production of Single Cell Protein from pineapple using yeast. *Innovative Romanian Food Biotechnology*. 8: 26-32.

Enwefa, C. (1991). Biomass Production from banana skins. *J. of Applied Microbiology and Biotechnology* 36(2): 283-284.

Han, V.W., (1975). Microbial Fermentation of Rice straw: Nutritive composition and in vitro digestibility of fermentation products. *Applied Microbiology*, 29(4): 510-514.

Khan, M.Y., Dahot, M.U. and Khan, M.Y. (1992). Studies on single cell protein by *Aspergillus niger* in solid state fermentation of rice bran. *Journal of Islamic Acid Sci*. 5: 39-43.

Maini, S.B. and V. Sethi, (2000). Utilization of fruits and Vegetables processing waste. *Industrial publishing company. New Delhi. India.*

Martin, M.S. and I.C. Macsae, (1993). *Rhizopus oligosphorus* grown on natural rubber waste serum cell for the production of single cell protein: A preliminary study. *World Journal of Micro and Biotechnology* 8(1): 63-64.

Moharib, S.A. (21003). Utilization of Guava peel wastes as substrates for protein and proteasae production by *Saccharomyces cerevisiae*. *Advances in food Sciences*. 25(2): 49-55.

Nasir, M. and Butt, M.S. (2011). Maize Germ: A Nutrient Dense Substance for food value addition. *LAP Lambert Academic Publishing, Germany*. 240p.

Najafpur, B.D., (2007). Single cell Protein. Biochemical Engineering and Biotechnology: 332- *African Journal of biotechnology*.341.

Ojokoh, A.O. and R.E. Uzeh, (2005). Production of Saccharomyces Cerevisiae biomass in papaya extract medium. 4(11): 1281-1284.

Riviere, J. (1977). Microbial Proteins in Industrial Applications of Microbiology. *Surrey University Press*. 4 pp 105.

SAS (Statistical Analysis System), (1996): Personal Guide. *SAS Inc. USA*.

Saquido, P.M.A., Cayabyab, V.A., Vyenco, F.R. (1981). Bioconversion of banana wastes into single cell protein. *J. Applied Microbiology: Cambridge University Press*. 7th edition.

Schlegel, (1999). Studies of the activities and usefulness of microorganisms in the production of Single Cell Protein (S.C.P.): *General Cambridge University Press.* 7th edition.

Singh, B.D. (1998). Biotechnology: 1st Edition. *Kalyani Publishers, New Delhi.* Pp 500.

Yabaya, S. and S.A. Ado, (2008). Mycelial protein production by production by *Aspergillus niger* using banana peels. *Science World Journal* 3(4): 9-12.

Table 2: MEDIA ANALYSIS OF SINGLE CELL PROTEIN

Characteristics	Pineapple wastes	Saw dust	Rice bran	Brewer's waste
Moisture %	82[a]	46[bc]	43[c]	56[b]
Reducing sugar	9.90[a]	3.80[a]	3.60[a]	4.20[a]
None reducing sugar %	11.50[a]	5.20[a]	4.80[a]	9.60[a]
Crude Protein %	0.80[a]	0.40[a]	0.32[a]	0.53[a]

Mean with different alphabet along the same row are significantly different ($P \geq 0.05$)

Table 3: THE WET AND DRY BIOMASS OF SINGLE CELL PROTEIN

Single Cell Protein	Wet Biomass %	Dry Biomass%
Pineapple	3.03	0.89
Saw dust	2.70	0.83
Brewer's	2.60	0.91
Rice bran	2.83	0.98

Table 4: PERCENTAGE BY WEIGHT OF BIOMASS OF SINGLE CELL PROTEIN

Characteristics	Pineapple wastes	Saw dust	Rice bran	Brewer's waste
Moisture %	70.62[a]	69.25[a]	65.37[a]	65[a]
Crude protein %	22.65[b]	6.45[a]	4.50[a]	11.6[a]
Reducing sugar %	10.20[a]	3.80[a]	3.25[a]	5.68[a]
None Reducing sugar %	30.0[a]	15.6[bc]	12.6[c]	28.8[ab]