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Use of Agrowaste (Cassava Peels) to Cultivate *Aspergillus niger* for Biomass Production

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Abstract Clinical strain of *Aspergillus niger* was cultivated using un-supplemented cassava peels to produce fungal biomass in this work. The proximate contents and organoleptic properties (smell, taste, viscosity, colour) of both the fermented and unfermented cassava peels broths were investigated adopting known methods. The total biomass produced was also established through a period of nine days. The result obtained showed the moisture content of the broth reduced from 86.29% to 31.60% at the end of the experiment. Similarly, crude fibre reduced from 10.88% to 8.87%. Also, the starch content reduced from 56.72% to 20.09% and cyanide reduced from 118.86ppm to 20.46ppm. On the other hand, Protein content increased from 6.24% to 11.22% and Ash content also increased from 4.88% to 10.23%. Biomass production increased daily from day one with initial weight of 1.253gm to 6.043gm on the 4th day. The biomass production peaked at day 5 with a weight of 8.266 gm and gradually reduced until a constant weight of 1.890gm was obtained on days 8 and 9. The quantity of biomass produced within the period of study makes the medium; cassava peels a good materials for biomass production which can be used in some industries as raw materials.

Keywords: Agrowaste, *Aspergillus niger*, Biomass, Cassava Peels, Cyanide, Single Cell Protein

1. Introduction

A considerable quantity of by- products, wastes and cast offs are generated from the agro sector. These wastes if not properly disposed can accumulate in the environment and cause pollution [1]. The use of Agro-industrial based wastes in fermentation processes has been source of interest as a result of the low cost and ready availability. They are also environmentally friendly and are alternative sources of products like biogas, biofuel, mushroom, antibiotics, vitamins, antioxidant, animal feeds [2], as medium for fungal cultivation [3] and compost [4] amongst others. A large amount of national budget that could have been spent on social development are being used for fuel importation. This leads to poor infrastructural development. The generation of energy from biomass produced from agricultural waste will help reduce the dependence on fossil and can also negate the effect of social and environmental impacts such as unemployment and global warming [5]

The accumulation of wastes can lead to environmental pollution resulting in health crises as well as the distortion of normal soil microbiota, leading to poor quality produce [6]. [7] opined that the changing economic, social, political and cultural values of the world has prompted many countries to look inwards and exploring effective means of waste disposal using industrial biotechnology. The agro- industrial waste contains large amounts of nutrients which are essential for growth and proliferation of microorganisms and subsequently the synthesis of useful products like enzymes [8], energy production [9], biomass production [10] and organic acid like lactic acid, butanol, methanol and ethanol [11].

Agro- industrial waste utilization is on the upward rise and is expected to continue rise to be able to sustain the ever increasing population of the world. Most agricultural wastes have composition rich in sugars, minerals, proteins and phytochemicals and therefore, cannot be referred to as wastes, but rather as raw materials for other industrial processes. The nutrients and moisture content of these wastes provides

conditions suitable for the growth and development of microorganisms. The ability of microorganisms to biodegrade agro products depends their enzymatic composition and this open up great possibilities for their use in fermentation processes. Microorganisms have unique properties required for bio- recycle that can drive environmental nuisance to zero waste. The use of agro-industrial wastes in fermentation processes is of interest due to their availability and low cost, they are also environmental friendly by reducing pollution and their ability to be recycled to make better secondary products [12]. Due to it's large availability and rich composition, agrowaste can be used in other processes. There is a great interest in the use of agricultural waste both from economic and environmental points of view. The economical aspect is based on the fact that such wastes may be used as low-cost raw materials for the production of other improved compounds, with the expectation of reducing the production costs while from the environmental point of view, reduces air pollution [13] and for generating energy including generating electricity, heating homes, fueling vehicles and providing process heat for industrial facilities [14].

Biomass is a renewable organic materials from plants and animals. Biomass energy is of great interest in both developing and developed countries because biomass alleviates dependence on limited fossil fuels, create employment opportunity and also develops the economy and revitalizes the rural communities. Biomass is a clean source of energy and reduces air emission than fossil fuels. Biomass energy is renewable and therefore sustainable [15]. The potential of microbes in alternative fuels and energy production is still largely unexploited. The need to explore single cell organisms in harvesting energy is a course that should vigorously pursued.

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Cassava (*Manihot esculenta* Crantz) is a staple African food that is widely consumed all over the world. But the waste produced thereof are been discarded and fed to animals in raw form with the high cyanide content. Large amount of the agro-industrial wastes are mainly composed by cellulose, hemicellulose and lignin, being called "lignocellulosic materials". Cassava wastes, peels, leaves and starch residues make up 25% of the total cassava plant [17]. The use of cassava peels is minimal due to the high content of cyanide and fiber as well as low protein and therefore is disposed off after cassava processing into food or other industrial products [18, 19]. The use of cassava peels as an energy producing raw materials is a means of limiting environmental degradation and energy paucity issues, as well as minimizing the health problems associated with environmental pollution as a result of the combustion of firewood and charcoal in cassava producing nations.

Microorganisms have been good candidates in agrowaste conversion and this has made them alternatives for high-input farming practices [20]. *Aspergillus niger* is useful in the industrial production of citric acid, gluconic acid and have been assessed as acceptable for daily intake by the World Health Organization [21]. *A. niger* fermentation is "generally recognized as safe" (GRAS) by the United States Food and Drug Administration under the Federal Food, Drug, and Cosmetic Act [22]. Other useful enzymes produced by A, niger include; Glucamylase, pectinase, Alpha galactosidase, glucose oxidases and cortisone.

Biomass produce by *Aspergillus niger*, is a byproduct of citric acid fermentation that has been a valuable material to many industries. Aspergillus niger degrades plant biomass polysaccharide to monomeric sugars, transports them into its cell and uses catabolic pathways to convert them to biochemical building blocks and energy [23].

Aspergillus niger is a ubiquitous filamentous ascomycete fungus, which efficiently degrades all plant polysaccharides through a wide range of extracellular carbohydrate acting enzymes (CAZymes). The Aspergillus group can, once cultivated, be used to synthesize useful industrial compounds such as 'glycoside hydrolases'. These enzymes are used in the process of converting biomass into biofuels – by breaking down cellulose and hemicellulose from plant cell walls, into a substance which is later converted into second-generation ethanol. This organism can also be used to produce bioactive metabolites, as well as other pharmaceutical products. In this work, the ability of *A. niger* to produce considerable biomass in cassava peels broth is investigated.

2. Materials and Methods.

2.1. Collection and Preparation of Sample

Cassava waste peels were collected from cassava mills in Owo metropolis. The peels were collected in clean polyethylene bags and taken into the laboratory for further analysis. The peels were washed thoroughly under running tap to remove sands, dirt and other unwanted materials. The cassava peels were then dried in the oven at 55°C for 14 days until crisp. The high temperature was to reduce the cyanide content. The dried peels were then pulverized into powder using the laboratory blender. The powder was sieved to remove fibres and particles.

2.2. Collection and Maintenance of Aspergillus Niger

Aspergillus niger spores were collected from slants in the microorganism bank of the Microbiology Laboratory of Achievers University, Owo. Ondo State. The spores were used to inoculate PDA slant and the slant allowed to incubate

and kept until use.

2.3. Inoculation of Cassava Peels Broth

0.5grms of the fungal spore was introduced into 10 mls of PDA broth and allowed to incubate for 5 days at 28°C. After this the *A. niger* culture was dispensed into 1000ml of cassava peels broth that was already prepared in a 2 ltr volumetric flask. The flask was plugged with cotton wool and allowed to stand for fermentation to commence. Samples were taken daily for proximate analysis and biomass content.

2.4. Proximate Analysis of Cassava Peels

The Proximate analysis of the cassava peels was carried out to determine its nutritional compositions. Ash content was determined using the method of [24], crude fibre content was determined by the tricyclic acid TCA method (IITA) [25], moisture content was determined according to the oven method of AOAC [26], fat content was determined using the Soxhlet extraction, protein content was determined by the Micro-Kjeldahl method and carbohydrate content was given as total carbohydrate by difference.

2.5. Total Cyanide Determination

The cyanide contents of the fermented cassava peels were also determined using the method of (27). Total cyanide (cyanogenic glycosides +cyanohydrin + HCN): In a stoppered 1.5 ml tube 0.1 ml extract and 0.05 ml linamarase were added to 0.45 ml phosphate buffer pH 7.0. After incubation at 37°C for 30 min, the mixture was transferred to a 15 ml tube containing 0.6 ml 0.2M NaOH. After 5 min, the sample was diluted with additional 2.8 ml phosphate buffer (pH 6.0) and analyzed in the spectrophotometric procedure.

2.6. Preparation and Sterilization of Medium

500gm of powdered cassava peels was measured into a 1ltr volumetric and 750ml of water was added. The solution was boiled on the hot plate for about 15 minutes and the allowed to cool. The solution was then filtered and the filtrate collected was autoclaved at 121°C for 15minutes. The sterilized medium was left overnight to cool. A loopful of *Aspergillus niger* on agar slant was used to inoculate the sterile medium and the medium stirred vigorously to allow for the dispersion of the spores of the fermenting organisms.

2.7. Fermentation of Cassava Peels Broth by Aspergillus Niger

Cassava peel broth prepared as described in 2.5, was inoculated with *Aspergillus niger* under sterile condition and was allowed to stand for 10 days under aerobic condition at a temperature of 30°C. The pH were monitored daily until the completion of fermentation and the values recorded. The proximate analysis were carried out before and after fermentation and the values recorded.

2.8. Physico-chemical Analysis

The physic-chemical analysis like colour change, appearance, turbidity, smell were monitored prior to fermentation and daily for the duration of the fermentation and the values recorded.

2.9. Determination of Biomass Content

The biomass was determined at intervals of 24 hrs. The suspension was filtered daily and the broth filtered through Whatman No. 1 filter paper to harvest the mycelia. The filter cake paper was washed thrice with deionised water, dried at 105°C to a constant mass in an oven (Gallenkamp), and weighed as the biomass. The day a constant weight was achieved, the experiment was terminated.

2.10. Statistical Analysis

The values obtained in this work were subjected to statistical analysis using T- test.

3. Results and Discussion

The conversion of the agricultural waste to useful industrial raw material or energy yielding materials is of great importance especially in developing countries. Therefore most attention today must be given to possible use of microorganisms to convert relatively high-energy wastes into more useful and highly nutritious end product. However, there are some important considerations necessary for microbial conversion i.e. which microorganism or microorganisms possess potentials for the bioconversion of the organic materials under consideration

3.1. Proximate Content of Cassava Peels

The result of the proximate analysis of the fermented and unfermented is presented in Table 1. The moisture content was 86.29% at the start of experiment but reduced to 31.60% after the experiment. This is contrary to the work of [27] who observed an increase in moisture content of their medium which he attributed to the combined activities of the microorganisms present in the medium.

The ash content increased from 4.88% to 10.23%. The increase in ash content could be suggestive of microbial activity. This agrees with the work of [28] who reported an increase in ash content in cassava products subjected to *Saccharomyces cerevisae.* The increase in ash content could also be attributed to the increase in mineral content, according to Oboh, [2006] increase in mineral content of a medium could be related to the loss of dry matter during microbial fermentation as microorganisms degrade carbohydrates and proteins. Protein content increased from 6.24% to 11.22% [29]. This increase could be attributed to the possible secretion of some extracellular enzymes (proteins) such as amylase, linamarase and cellulose [30]. Also, the increase in the growth and proliferation of the fungi used in medium to form of single cell protein may possibly

account for the apparent increase in the protein content of the fermented peels [31, 32]. It could also be attributed to the fermenting organism's attempt to make use of the cassava starch as a source of carbon [33]. Apart from this, the increase in the growth and proliferation of the fungi in the form of single cell proteins may possibly account for the apparent increase in the protein content of the fermented peels broth. This opinion is supported by the works of Akinfala and, Tewe [2004]; Tweyongyere, and Katongle, [200] s [34, 35].

The fat content increased from 1.39% to 2.91%. The increase in the fat content might due to the increase in the microbial mass, activities of lipolytic microorganisms to secrete extracellular enzyme (lipase), secretion of microbial oil into the fermenting medium and other products from the metabolism [36]

Crude fibre of the unfermented broth reduced from 10.88% to 8.87% in the fermented broth. The decrease observed could be attributed to the ability of the *Aspergillus niger* to degrade the crude fibre in the fermenting cassava peel, secrete hydrolyzing and oxidizing enzymes involved in the conversion of recalcitrant compounds in the waste into utilizable compounds (35) and abundant production of organic acids resulting from fermentative dissimulation of carbohydrates [37].

The significant decrease ($P < 0.05$) in the carbohydrate content of the fermented cassava peels broth when compared to the unfermented broth (Table 1) could be attributed to the ability of the fungi complex to hydrolyze starch into glucose which may ultimately have been used by the organisms as a carbon source to synthesize biomass rich in protein. The proportionate increase in the protein content of the fermented peels could also have accounted for the decrease in the carbohydrate content (35).

Table 1. *Proximate analysis result of unfermented and fermented cassava peels.*

Composition	Unfermented	Fermented
Moisture content	86.29	31.60
Ash	4.88	10.23
Protein	6.24	11.22.
Fat	1.39	2.91
Crude fibre	10.88	8.87
Cyanide	118.86ppm	20.46ppm
Starch	56.72	20.09

3.2. Cyanide Content

There was a significant decrease in the cyanide content of the cassava peels broth at the end of experiment as compared to the broth before biomass production (Table 1). This reduced value could be attributed to the fact that *Aspergillus niger* was capable of partially degrading the cyanogenic glycosides in the peels. This is in line with the reports of Yabaya, and Ado [2008] and Okpako [2008] [25, 38]. The reduction might also be due to the synergistic effect of loss of cyanogenic glycosides on hydrolysis by linamarase [39], metabolic activities of inherent microorganisms [27, 40], ability of the microorganisms to secrete extracellular enzymes (amylase, xylanase and linamarase), increase in cell mass and formation of a hydrolytic complex bind force to the cyanide compound.

3.3. Biomass Production

Biomass content peaked on the fifth day of experiment at 8.266gm. However, the biomass decreased on daily basis until a constant weight of 1.890gm was obtained on the 9th and 10th day (Table 2).

The high yield of mycelia or biomass of *A. niger* using cassava peel broth and the persistent growth of the organism might be due to the presence of nutrients in the broth. The stability of the growth could also be as a result of the presence of cellulose in the substrate. Cellulose, starch, and pectin were likely to have been broken down to glucose and other sample sugars by carbohydrate hydrolyzing enzymes such as cellulase, amylase and pectinase known to be produced by *A. niger.* [41]. The decrease in biomass production after day 5 was due to decline in the available carbon and energy sources for the metabolic processes.

The decrease in biomass reported in this work is similar to the report of Muhammad et al. who reported maximum cell biomass of *A. niger* after 120h and *Penicillium javanicum* after 144h of fermentation. The reduction in biomass production can also be attributed to non- development of hyphae as a result of the depletion of nutrients in the medium.

Table 2. *Biomass production by Aspergillus niger in cassava peels broth.*

Time in minutes	Weight in grams
1	1.253
2	2.567
3	4.792
4	6.043
5	8.266
6	3.899
7	2.067
8	1.890
9	1.890

3.4. Organoleptic Qualities of Cassava Peel Medium Before and After Fermentation

The cassava peels broth was tested for smell, taste, colour, turbidity, and acidity both before and after fermentation. Considerable changes were observed on physical examination. These changes are as a result of fermentation processes. At the start of experiment, the broth was brown but as fermentation proceeded, the broth turned deep brown. The clear liquid at the start of the experiment became cloudy and highly turbid at the expiration of the fermentation. The increase in turbidity could be as a result of the accumulation of metabolic waste of the fermenting organism as well as the presence of hyphae in the medium. The taste was bitter with distinctive acidic smell (Table 3). The acidic fermentation smell agrees with the findings by Gonzalez [2007] who opined that a good fermentation smells like acid and is fragrant [43].

The reason for the change in colour of the medium could not be far from the fact that temperature during fermentation

causes Mailard's reaction that turns cassava peel brown [43].

Table 3. *Organoleptic characteristics of fermented and unfermented cassava peels broth.*

Parameters	Unfermented	fermented
Colour	Brown	Deeper brown
Odour	Acidic	Fermented odour
Viscosity	Light	light
pH	4.66	3.62
taste	Bitter and tangy	Slightly sweet
turbidity	Clear	turbid

3.5. pH of Both Fermented and Unfermented Cassava Peels Broth

The pH of the fermenting medium at the start of experiment was 4.66 but reduced to 3.62 at the end of the experiment. This is an indication of the acidic end product of the fermentation process became more acidic as *A. niger* breaks down the carbohydrate in the medium to produce organic acid. This is in accordance with Balagopopal and Maini [1976] who observed a decrease in pH during fermentation of sweet potato. [45]

A. niger has been reported to produce an array of extracellular enzymes required for the degradation of the major components in agricultural wastes. [46] Femi-Ola*, et al.,* [2013], reported that *A. niger* was superior to other species of Aspergillus and Rhizopus in amylase production. [46]. Hang et al., [1975] also reported a high biomass production in their work using brewer's spent grains to cultivate fung i [47]. The enzymatic activity of this organism and the rapid breakdown of the substrate may be responsible for the biomass produced by *A. niger.* Ikenebomeh and Chikwendu, [1997] reported a dry biomass of 13g/L of *A. niger* grown in brewery spent grain liquor [48]. Hang, *et al*., {1975] observed the dry biomass production of 15.60g/L of *A. niger* when cultivated using cassava rind basal medium [47]. Lower biomass of 9.82± 0.35g/L was observed using cassava whey [48].

4. Conclusion

Cassava peels fermented with *Aspergillus niger* produced materials with reduced cyanide level compared the unfermented peels. *Aspergillus niger* can be a good candidate for the large scale production of mycelial protein using cassava peels as the substrate. Cassava peels which are regarded as having no economic value could be engineered by microorganisms to increase nutritive value, reduce cyanide content and used as feed for farm animals provided it is accepted especially since the fungi has broken down the complex molecules, especially the fibre to become highly digestible. Furthermore, the need to explore organic waste materials as alternative source of microbial growth medium is essential due to an ever increasing high cost of substrate for microbial cultivation. The potential of the moulds to utilize agrowastes as substrates could be harnessed for effective waste management. It is recommended that the study on mycelia protein production by *A. niger* using cassava peels should be conducted on a large scale.

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