Global Journal of Applied, Management and Social Sciences (GOJAMSS); Vol.24, April 2022; P.172 – 182 (ISSN: 2276 – 9013)

STUDIES ON THE USE OF CASSAVA EFFLUENT AS SUBSTRATE FOR BIOMASS AND AMYLASE PRODUCTION FROM *ASPERGILLUS NOMIUS*

UMEODUAGU, N.D.
Department of Applied Microbiology and Brewing,
Nnamdi Azikiwe University, P.M.B. 5025 Awka, Nigeria.

&

ODIBO, F.J.C.
Department of Applied Microbiology and Brewing,
Nnamdi Azikiwe University, P.M.B. 5025 Awka, Nigeria.

&

IKELE, M.O.*
***Corresponding author's e-mail: mo.ikele@unizik.edu.ng; ORCID: 0000-0001-7628-2962**
Current Address: Department of Microbiology,
Tansian University, P.M.B. 0006 Umunya, Nigeria.

Abstract
Cassava waste water is a starch-rich effluent produced from cassava bio-processing which is discarded into the environment where it constitutes pollution. This research aim was to produce biomass and amylolytic enzyme from Aspergillus nomius using cassava effluent. The effluent was obtained from local gari processing plants in Awka, Nigeria. Physico-chemical components of the waste water were determined. Fungal biomass was produced from 500 ml enriched waste water in a rotary shaker. Amylase was produced from the waste water in a batch fermentation using the best nitrogenous source fortification and its time course was monitored. Amylase and protein were assayed using standard procedures at 540 nm and 280 nm, respectively.The produced crude enzyme was tested for relative rate of raw starch hydrolysis on different native food starch substrates. The waste water had a starch content of 87.575±3.03 mg/L and reducing sugar of 129.3 g/L. A biomass weight of 3.9 g/100 ml of optimized waste water medium was recovered with an amylase activity of 4.9 u/ml. Yeast extract (1.5% w/v) gave the highest amylase activity compared to other nitrogenous sources. Recovered crude enzyme gave a percentage relative raw starch hydrolysis rate of 100 %(millet), 91.6% (Aerial yam), 87.5% (cassava) and 75% (wild cocoyam). This study shows that cassava waste water can be re-cycled as a useful and cheap industrial raw material for sustainable production of fungal biomass and amylase for industrial applications.

Key words: Amylase, *Aspergillus nomius,* Agro-Waste Conversion, Cassava effluent

Introduction

Environmental pollution is one of the most dangerous hazards affecting both developing and developed countries, as a result of industrial growth, urbanization and man-made problems resulting from population growth (Adewumi and Oguntuase, 2016). The main sources of environmental pollution are agricultural effluents, industrial effluents, sewage, fossil fuel plants and nuclear power plants. Starchy and ligno-cellulosic wastes are main agricultural wastes in many tropical countries, which include Nigeria. Cassava (*Manihotesculenta*Crantz), is a tropical crop consumed on daily basis in Nigeria. It has a wide extent of use in the country, ranging from fufu to *gari,* which are mainly taken with soup; to cassava flour and starch which are used for a variety of other purposes (Uguru and Obah, 2019). Due to the diverse advantages of

cassava, there is constant economic use for it, which leads to constant cassava processing going on in several cottage industries around the country. Cassava wastes generated from these industries are one of the most frequent agricultural wastes found in the country, whether in form of pulp, bark or effluent. Out of these three major wastes generated from cassava processing, the pulp and bark are quite decomposable when disposed into the environment, but the waste water introduces harmful substances such as nitrogenous compounds and cyano-glycosides that are hydrolyzed into hydrogen cyanide, which is toxic and pose serious threat to the environment (Agbo*et al*., 2019; Uguru and Obah, 2019). Improper effluent discharge experienced around the country is bordered on two main factors which are; lack of adequate and workable drainage systems, as well as lack of technical expertise on waste water treatment by the operators of these industries (Adewumiand Oguntuase 2016; Agbo*et al*., 2019). In other words, most cassava processing industries are sited across the nation, with little or no emphasis made on proper drainage systems and efficient safe effluent discharge. Thus, effluent discharge becomes the chief culprit in environmental pollution from cassava bio-processing industries. One of the ways to manage cassava effluents, in order to reduce environmental contamination with the toxins they contain, is by converting the effluents to useful substrates for culturing microorganisms of industrial importance, that possess the capacity to utilize or degrade the toxins contained in them.These microbial biomass are important for proper effluent bio-degradation and also for production of useful metabolites like enzymes of industrial importance.

Amylases are enzymes that catalyze hydrolysis of glycosidic linkages in starch components or related carbohydrates, releasing malto-oligosaccharides and glucose in the α or β anomeric form (Kalairasi and Palvartham, 2013). Amylolytic enzymes are the most important industrial enzymes which can be used in a number of industrial processes including brewing, baking, textile, detergent, conversion of starch to sugar syrups, production of cyclodextrins, preparation of digestive aids, production of chocolates, cakes, fruit juice *inter alia*. Besides their use in saccharification, they also find application in paper and distillery industries (Martin *et al*., 2019).

A lot of microorganisms have been employed in the microbiology of bio-degradation of toxic substances from different environmental problem-creating substrates. Microbes such as *Aspergillus, Pseudomonas, Bacillus* amongst others have been reported to have good bio-degradation ability, and apparently, their biomass are greatly needed and useful for this purpose (Abdulmajeed *et al*., 2016). Amongst the species of *Aspergillus*; *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus* have been extensively studied for environmental bio-remediation (Abdulmajeed *et al*., 2016).*Aspergillus nomius* is a species related to *Aspergillus flavus* and *Aspergillus tamarii* (Kurtzman *et al.*, 1987; Handayani *et al*., 2019). Despite the fact that *A. nomius* produces B-class aflatoxin (which are harmful to man), a few studies carried out on it has shown it to be useful to man through their ability to produce different microbial enzymes that have been used for industrial purposes (Olsen *et al*., 2008; Sun *et al*., 2009; Handayani *et al*., 2019), thus, this research adopted *A. nomius* as a novel approach for cassava waste water conversion as substrate for producing value-added product like amylase.

Methods

Physico-Chemical Analyses of Cassava Waste Water

Determination of total solids in the waste water

This was carried out using the method described by APHA (1998).

Determination of total suspended solids

Total dissolved solid was first determined using an automated TDS machine (Tester model 480). The tester probe was dipped into 50 ml of sample and the result was read from the display unit of the machine. Thereafter, the difference between the total solids and total dissolved solids was calculated to be the total suspended solids i.e

TSS = TS – TDS.

Where;
Total dissolved solids (mg/L) = (A-B) x1000
Volume of water used
A= weight of solids + filter paper
B= weight of filter paper

Determination of pH, Reducing Sugar, Starch and Protein Contents
The pH, reducing sugar, starch and protein contents of the waste water were determined as described by APHA (1998).

Isolation and Characterization of *Aspergillus nomius*
A 200 ml aliquot of the effluent sample was obtained and diluted using one in ten-fold serial dilutions in a sterile Sabouraud's Dextrose broth. Thereafter, 0.1 ml of the diluted sample was cultured on Sabouraud's Dextrose agar and incubated aerobically at 28°C for 48 h. *Aspergillus* sp. were identified presumptively using a fungal atlas and confirmatory test was done using Inter-specific region sequencing of the amplified rDNA.

Enrichment of Cassava Waste Water for Biomass and Amylase Production
This was conducted using modified method described by Oshoma *et al*. (2009). Cassava waste water was enriched by the addition of 1.5 % peptone, 0.3 % NaCl, 0.3 % KH_2PO_4, 0.1 % $MgSO_4.7H_2O$ for biomass growth, while 0.3 % yeast extract was added in place of 1.5 % peptone in 500 ml cassava waste water for enzyme production, in Erlenmeyer flasks and autoclaved. Agar plugs of the fungi were introduced into the enriched medium and were incubated at 30°C in a rotary shaker for ten days. The contents of the Erlenmeyer flasks were filtered using pre-weighed Whatman No. 1 filter paper. The filter paper content was dried and total biomass produced was determined by the calculation;

Biomass = $W_2 – W_1$
Where W_1 = weight of empty filter paper
W_2 = weight of dried biomass and filter paper.

Assay of Amylase
This was determined using a modified version of the method described by Umeh and Odibo (2014). A 1 ml aliquot of crude enzyme was added to 1ml of standard starch solution (1% w/v soluble starch and 0.006 M NaCl in 0.2 M Phosphate buffer pH 6.9) and incubated at 40°C for 30 minutes. Reducing sugars released by the enzyme were determined by adding 2 ml of dinitrosalicyclic acid (DNS) reagent, boiled for 10 mins and then cooled under running tap water. Ten millilitres of distilled water was then added and allowed to stabilize for about 5mins. The absorbance of the resulting solution was determined at 540 nm with a spectrophotometer against a reagent blank. One unit of amylase activity is taken as the amount of enzyme in 1ml of crude amylase that produced 1.0 mg of reducing sugar per mol of starch under the assay conditions.

Effect of Nitrogenous Sources on Amylase Production Using Cassava Waste Water
The effect of the following nitrogen sources on amylase production was investigated: soybean meal, bacteriological peptone, ammonium sulphate, yeast extract and groundnut cake. The cassava waste water was fortified with 1% of each nitrogen source and the following mineral salts: 0.3% NaCl, 0.3% KH_2PO_4 and 0.1% $MgSO_4.7H_2O$. Flasks containing 50 ml of medium each were inoculated with 1 ml of spore suspension of *A. nomius* and incubated at 30°C in a rotary shaker at 200 rpm. Amylase activity (u/ml broth) was determined 24 hourly until 96 h.

Effect of Yeast Extract Concentrations on Amylase Production
The effect of different concentrations (0.5-4%) yeast extract on amylase production in cassava waste water was determined. Fermentation was carried out as described but for 48 h and the amylase activity determined using DNS.

Time Course of Amylase Production using Fortified Cassava Waste water
The time course for amylase production was studied by growing *Aspergillus nomius* in a medium consisting of cassava waste water (87 mg/ml starch content), 1.5% yeast extract, 0.3% NaCl, 0.3% KH_2PO_4 and 0.1% $MgSO_4.7H_2O$. Fermentation was carried out in a shaker at 30°C and 300 rpm in 250 ml Erlenmeyer flasks each containing 50 ml of broth. Enzyme activity (u/mol), culture pH and biomass were monitored daily until maximum enzyme yield was obtained.

Hydrolysis of Raw Starch
Bicolor wild cocoyam, cassava, millet, and aerial yam (*Dioscorea bulbifera*) were processed using the method described by Obi and Odibo (1984). Briefly, wild cocoyam, cassava and yam were first peeled and reduced to pulps using a hand grater. Subsequently, the pulps and the millet grains, after soaking for 1 h in water were separately homogenized in a waring blender. From each homogenate contained in a bag of fine white cloth, starch was leached into a plastic vat by churning with excess water. The crude starch suspension in the vat was allowed to settle overnight, after which the sediment was separated from the supernatant by decantation and dried at 50°C for 48 h. The resultant flakes were ground to a fine powder and used as native starches. To assess the hydrolysis of the starches by the amylase, one set of each starch was gelatinized by heating a suspension of each starch (1% w/v) in 0.1 M phosphate buffer pH 6.8, while the other set was suspended in buffer without heating. Aliquots (0.5 ml) of all raw starches were separately hydrolyzed with 0.5 ml of amylase at 40°C for 10 minutes and the reaction stopped with 1 ml of DNS. The optical density of the reaction mixture was determined at 540 nm.

Statistical Analyses
Data were analyzed using one way Anova with SPSS version 23.

Results
Physico-chemical Assessment of Cassava Waste Water.
Physico-chemical parameters of cassava waste water revealed a pH of 3.81±1.33, starch content of 87.857±3.03 mg/L , reducing sugar of 129.3 g/L and protein content of 11.20±2.52mg/L, total solids of 109.34±0.69, total dissolved solids of 42.90±1.05mg/L and total suspended solids of 66.44±1.28mg/L as shown in Table 1.

Isolation and Characterization of *Aspergillus nomius*
The presumptively characterized *Aspergillus* species chosen for the study was confirmed with molecular typing as *Aspergillus nomius* (gi:356614401).
***Aspergillus nomius* Biomass Production Using Optimized Cassava waste Water Medium**
Biomass production using optimized waste water medium gave a highest wet biomass value of 3.9 g by day 6 of cultivation at pH 8.2 as seen in Figure 1. Dry biomass weight measured gave the highest value of 1.9 g at same pH and day of cultivation as shown in Figure 2. The percentage biomass yield was calculated to be 40.91 % as shown in Table 2. Assay of amylase activity from the crude enzyme gave highest value of 4.9 u/ml at pH 8.2 at day 4 as shown on Figure 3.
Effect of Cassava Waste Water Fortification with Different Nitrogenous Sources on Amylase Production
Incorporating 1% (w/v) of different nitrogenous bases to the cassava waste water showed that yeast extract gave the highest amylase activity out of them all (Fig. 4). Precisely 1.5 % (w/v) of yeast extract gave the highest amylase production from *A. nomius* at 48 h (Fig. 5).

Time Course of Enzyme Production using Fortified Cassava Waste Water

The time course of the production of amylase of *Aspergillus nomius* is shown in Figure 6. Amylase production followed exponential growth of the organism with maximum enzyme occurring during the exponential (48 h) phase corresponding with the culture pH of 8.0.

Raw Starch Digestion Activity of Amylase

Percentage relative rate of hydrolysis of raw starches is in table 3.The highest rate (100%) of hydrolysis of raw starch by the enzyme was obtained with millet starch followed by aerial yam starch. Wild cocoyam starch was the least hydrolyzed.

Table 1: Physico-chemical Parameters of Cassava Waste Water

Parameters	Concentration
pH	3.81±1.33
Total Solids (mg/L)	109.34±0.69
Total Dissolved Solids (mg/L)	42.90±1.05
Total Suspended Solids (mg/L)	66.44±1.28
Reducing Sugar (g/L)	129.3±0.03
Starch Content (mg/L)	87.857±3.03
Protein Content (mg/L)	11.20±2.82

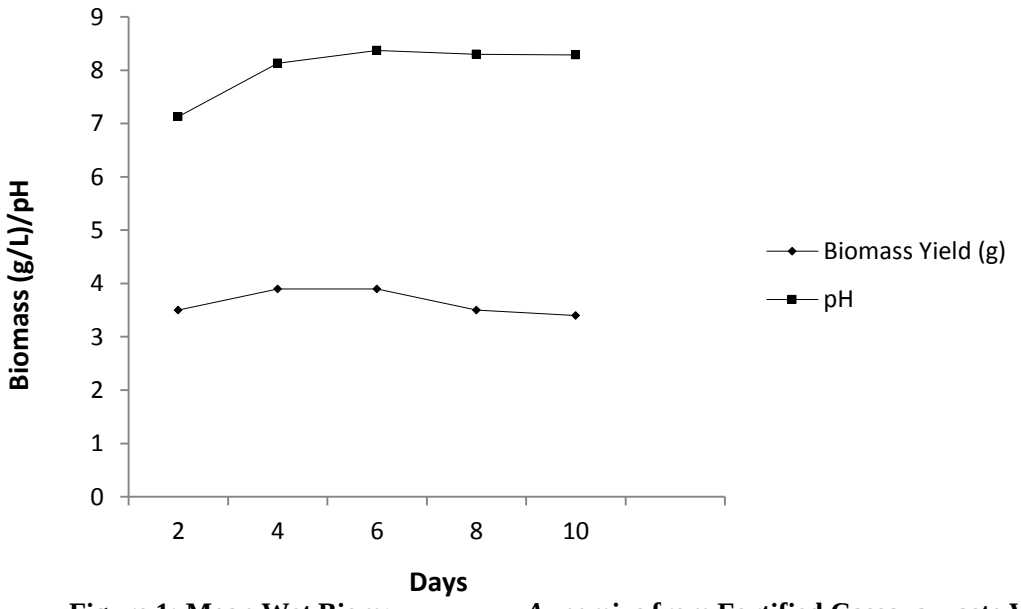

Figure 1: Mean Wet Biom*a* ***A. nomius*** **from Fortified Cassava waste Water.**

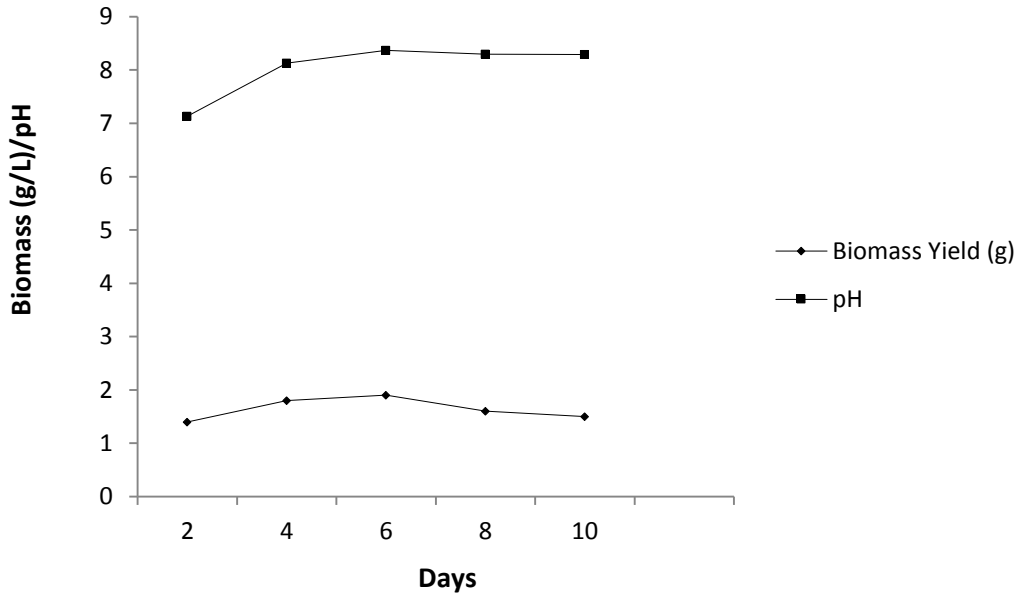

Figure 2: Mean Dry Biomass Yield of *A. nomius* in Fortified Cassava Waste Water

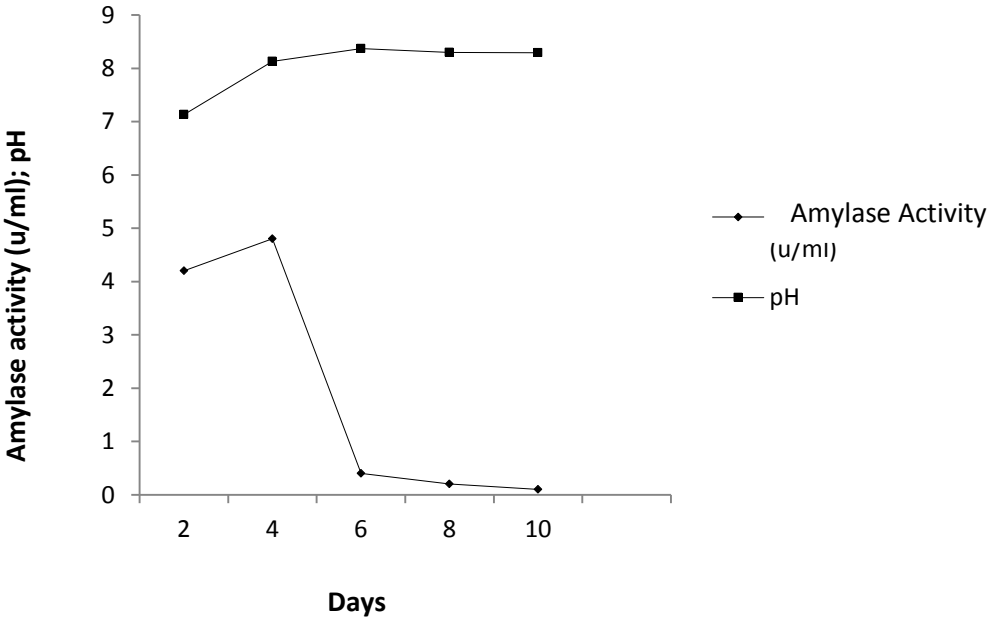

Figure 3: Amylase of *A. nomius* from Enriched Cassava waste Water

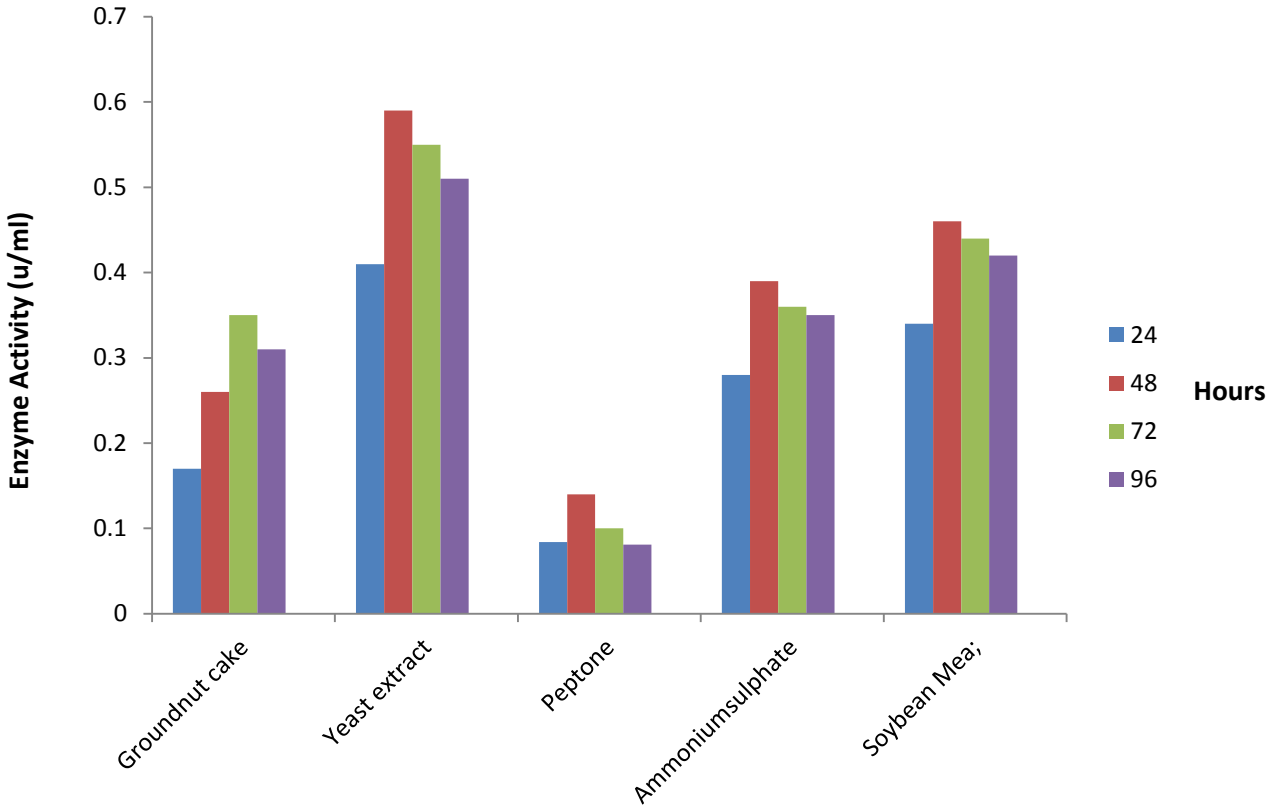

Figure 4: Effect of 1 % (w/v) Nitrogenous Substrate Fortification on Amylase Production

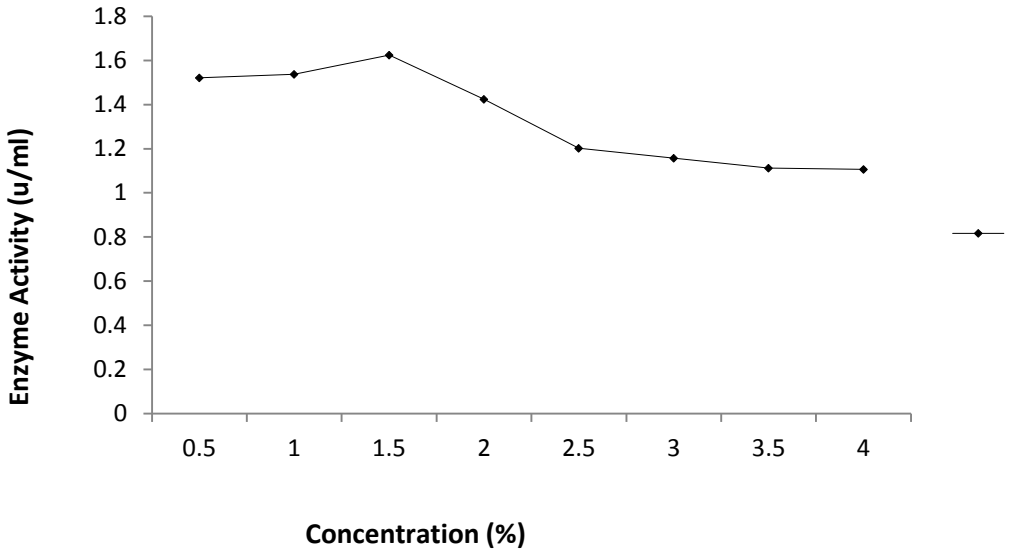

Figure 5: Effect of Yeast Extract Concentrations (% w/v) on Amylase Production at 48 hours

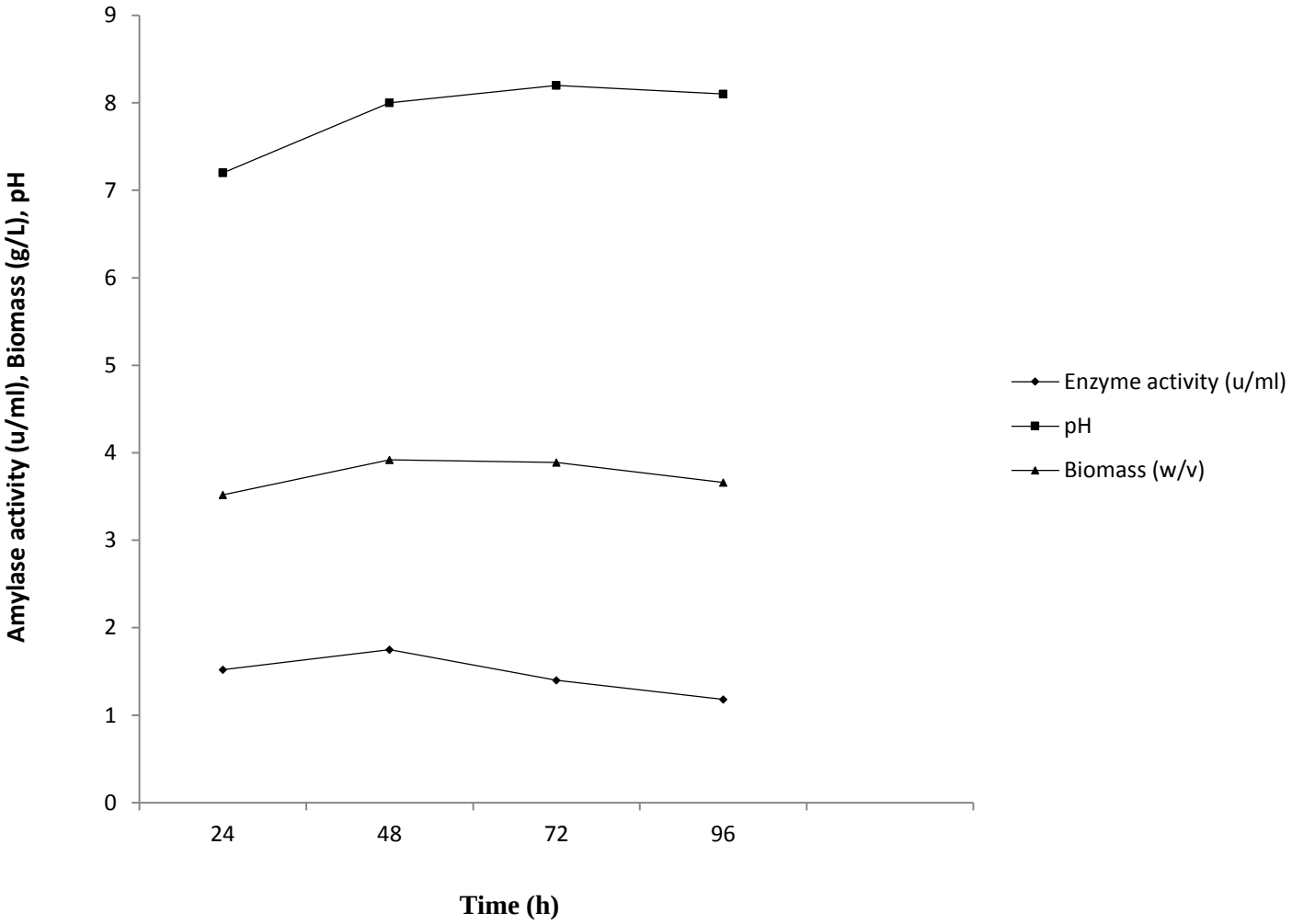

Figure6: Time Course of Enzyme Production using Fortified Cassava Waste Water.

Table3: Percentage Relative Rates of Hydrolysis of Starchy Substrates

Starch substrates	Raw starch (%)
Millet	100
Cassava	87.5
Wild cocoyam	75
Aerial Yam (*Dioscorea bulbifera)*	91.6

Discussion

Cassava waste water from *gari* processing plants was chosen as a substrate for the sustainable production of amylase from *Aspergillus* because of its abundance and availability, cheapness and high starch content.
The physico-chemical properties of the waste water show that it has a reducing sugar content of 129.3 g/L, starch 87.7 mg/L and protein 1.2 mg/L (Table 2) and thus can serve as a nutrient medium for the growth of microorganisms for the production of value added products. Adetunji *et al*. (2015) explained this to be as a result of residual nutrients from cassava roots during retting and washing. Umeh and Odibo (2014) stated that retting periods have effect on the nutrients that get into the steep water from the peeled cassava roots, which when eventually the retted roots are removed, residual nutrients abound in the waste water. Optimization of cassava waste water has been shown to be beneficial in the biomass growth of molds with industrial advantages. Oshoma *et al*. (2009) reported that optimization of cassava waste water gave a biomass yield of 1.63 g/l for *Aspergillus niger* with amylase activity of 495 u/ml at 5 days of cultivation. This study however, work gave a biomass yield of 3.9 g (40.91% yield), amylase activity of 4.9 u/ml, at 6 days of cultivation for *A. nomius*. Oshoma *et al*. (2009) reported yeast extract as the most favourable nitrogenous base to be supplemented with cassava waste water because it gave the highest biomass and amylase yield for *A. niger*. Kalairasi and Palvartham (2013) also reported yeast extract as the best nitrogenous base for amylase production by *Bacillus cereus* (MTCC 10202). Thesepreceding findings correspond with the findings in this study in which yeast extract at 1.5 % concentration also gave the highest amylase activity by*A. nomius* at 48 h (Figure 4 and 5).Two other locally sourced nitrogenous bases (groundnut cake and soya bean meal) were also incorporated into the waste water to serve as substrates for amylase production, and they also appreciably stimulated amylase production by *Aspergillus nomius*. Time course study using cassava waste water fortified with 1.5% yeast extract gave optimal biomass and amylase activity at 48 h. Comparing this time course study (Figure 6) with the initial enrichment time course study process (Figure 3), it can be seen that *A. nomius* had a higher biomass and amylase yield in 48 h (2 days) as against the initial 4 days. This thus, shows that increase in the yeast extract concentration from 0.3% to 1.5% improved amylase production of this microorganism, shortened the production time, with no statistical difference ($p>0.05$) existing in the biomass yields in both methods. It was observed that millet raw starch was the most hydrolyzed by *A. nomius* amylase. This implies that covalent bonds in millet raw starch are easily broken by the amylase, however, this was not same for wild cocoyam which gave the lowest relative hydrolysis rate. Omemu *et al*., (2005) explained that wild cocoyam starch has low relative rate of hydrolysis and the covalent bonds could be properly broken if the incubation time of this starch and amylase is prolonged. It is possible that the rates of hydrolysis of these starches by amylase of *A. nomius* could change if they are gelatinized.

Conclusion

This study has shown that cassava effluent is rich in nutrients and has more benefits than it being considered a waste product,and thus, can be re-cycled for microbial biomass and amylase production, hence, solving the issue of cyanogenic glycoside pollution it creates when discharged to the environment during cassava processing. It is a natural and cheap raw material generated on daily basis in several cassava processing cottage industries in Nigeria, so it can support sustainable production of useful *Aspergillus* biomass and fungal amylase.

Conflict of Interest

Authors declare no conflict of interests.

References

Abdulmajeed BO, Yusuf BA, Abdullahi IO (2016) Efficiency of *Aspergillus niger, Aspergillus flavus* and *Microsporum nanum* to remove Heavy Metals from Refinery Effluent. Journal of Advances in Biology and Biotechnology, 6(3): 1-6.

Adetunji AR, Isadare DA, Akinluwade KJ, Adewoye OO (2015) Waste-to-Wealth Applications of Cassava-A Review Study of Industrial and Agricultural Applications. Advances in Research, 4(4): 212-229.

Adewumi JR, Oguntuase AM(2016) Planning of Waste Water Re-use in Nigeria. The Journal of Sustainable Development, 15(1): 1-33.

Agbo BA, Ogar AV, Itah AY, Brooks AA, Akonjor MA(2019) Assessment of the Effects of Cassava Mill Effluent on the Soil and its microbiota in Biase Local Government Area of Cross River State, Nigeria. World Journal of Advanced Research and Reviews 01(02): 034-044.

American Public Health Association (1998). Direct Air-Acetylene Flame Method, Standard Methods for the Examination of Water and Waste Water, 20thedn. APHA.AWWA.

Handayani D, Artasasta MA, Taher M, Djamaan A (2019) Cytotoxic and Antibacterial Activities of Marine Sponge-Derived Fungus *Apergillusnomius* NC06. Rasayan Journal of Chemistry 12(3): 1462-1469.

Kalaiarasi K, Palvartham R (2013) Optimization of Process Parameters for Amylase Production under solid-state Fermentation by *Bacillus cereus* MTCC 10202. African Journal of Microbiology Research7(45): 5166-5177.

Kurtzman CP, Horn BW, Hesseltine CW(1987) *Aspergillus nomius*, A new Aflatoxin Producing Species Related to *Aspergillus flavus* and *Aspergillus tamari.* Antonie Van Leeuwenhoek53: 147-158.

Martin MF, Okpo EA, Andy E(2019) Microbial Amylases: A review. World News of Natural Sciences 22: 174-179.

Obi, S.K.C. and Odibo, F.J.C. (1984). Partial Purification and Characterization of a Thermostable Actinomycete β-amylase. Applied and Environmental Microbiology47(3): 571-575.

Olsen M, Johnsson P, Moller T, Paladino R, Lindbald M(2008)*Aspergillus nomius*, an important Aflatoxin Producer in Brazil Nuts? World Mycotoxin Journal 1(2): 123-126.

Omemu AN, Akpan I, Bankole MO, Tenida OD (2005) Hydrolysis of Raw Tuber starches by Amylase of *Aspergillus niger* Amo7 Isolated from the Soil. African Journal of Biotechnology4 (1): 19-25.

Oshoma CE, Imarhiagbe EE, Ikenebomeh MJ, Eigbaredon HE (2009) Nitrogen Supplements Effect on Amylase Production by *Aspergillus niger* using Cassava Whey Medium. African Journal of Biotechnology9 (5): 682-686.

Sun H, Xiangyang Ge, Wang L, Zhao P, Pery M(2009) Microbial Production of Raw Starch Digesting Enzymes. African Journal of Biotechnology8(9): 1734-1739.

Uguru H, Obah GE(2019) Remediation of Effluent from Cassava Processing Mills. Direct Research Journal of Public Health and Environmental Technology 4(4): 21-25.

Umeh SO, Odibo FJC(2014) Amylase Activity as Affected by Different Retting Methods of Cassava Tubers. International Journal of Agricultural Policy and Research2(7): 267-272.