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Studies on the biodegradation and utilization of selected tuber wastes by *Penicillium expansum*.

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ABSTRACT: Some fungi were found to be associated with the degradation of selected tuber starch wastes (Yam Peels, Cassava Peels, Sweet Potato Peels and Cocoyam Peels) of which eight showed amylase production on the Potato Dextrose Agar (PDA) supplemented with soluble starch. *Penicillium expansum* was selected among the isolates and used to ferment the extracts prepared from each of the tuber starch wastes for 144 hours. Yam peel was found to be highly susceptible to the degradation while cocoyam peels was most resistant to the degradation. Fermentation of each of the waste extracts showed the ability of the organism to increase the protein contents, amylase activities of the waste extract with the increase in biomass. However, concentration of sugar and starch reduced considerably.

Key words: Degradation, amylase, tuber starch wastes, peels, *Penicillium expansum*

Introduction

Carbohydrate-based agricultural products such as starchy tubers (Cassava, Yam, Sweet Potato and Cocoyam) are important staple foods in the diet of people in most developing countries of the tropics. In Nigeria, they are widely distributed than cereal (Akpan *et al.*, 2005). However, the generation of waste materials (peels) resulting from the production, preparation and consumption of food pose increasingly disposal and potentially severe pollution problems and represent a loss of valuable biomass and nutrients (El-Shimi *et al.*, 1987). These peels are regarded as waste and are usually discarded and allowed to rot. Vegetation and soil around the heaps of the peels are rendered unproductive and devastated due to biological and chemical reactions that take place between the continuously fermenting peels, soil and the surrounding vegetation.

Besides their pollution and hazardous aspects, in many cases, these peels might have potentials for recycling raw materials or for conversion into useful products of higher value or even as raw material for other industries or for their use as food or feed after biological treatment (Okolo *et al.*, 1995).

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Among the microorganisms, fungi had been found to be good sources of amylolytic enzymes (Akpan *et al.*, 2005). The present work, therefore, is aimed at monitoring the degradation processes and compositional changes of selected tuber starch wastes (peels) over a period of time for pollution abatement using a fungal agent.

Materials and Methods

Sources of samples

An underground layer of Cassava (*Manihot esculenta*) peels, Sweet Potato (*Ipomea batatas*) peels, Yam (*Dioscorea rotundata*) peels and Cocoyam (*Xanthomonas sagittifolium*) peels, which have started decomposing were obtained from different dumpsites in Ilorin, Kwara State of Nigeria.

Culture Media

The culture medium used for the isolation was Potato Dextrose Agar (PDA) supplemented with streptomycin to prevent bacterial growth. Serial dilution plate method was carried out on various decomposing tuber peels. The numbers of fungi on the plates with distinct growth were counted after incubation for 72hours. Fungal population was then estimated, recorded, identified and characterized.

Screening for Amylase Production

The method of Fawole and Oso (1998) was used. A point inoculation of the spores of the isolates were grown on PDA supplemented with 2% w/v soluble starch, incubated at 30°C for 48hours after which it was flooded with 2% Potassium Iodide solution. The production of extracellular amylase by the organism was indicated by a zone of clearance around the fungal colonies. The fungal isolate that occurred most with higher amylase production was selected and identified as *Penicillium expansum*

Substrate Pretreatment

The tuber starch wastes: Cassava peels, Yam peels, Cocoyam peels and sweet potato peels were washed aseptically and dried separately for 3hours in an oven at 70°C using a domestic blender (Moulinex Optiblend 2000 model AAW 941/711-10050). Muslin cloth was used to sieve and stored before use.

Culture Media Preparation.

The organism was cultivated in a mineral salt medium, each containing the following compositions (g/l): KH_2PO_4 , 10.0; $(\text{NH}_4)_2\text{SO}_4$, 10.5; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.33; CaCl_2 , 0.5; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.004; $\text{FeSO}_4 \cdot \text{H}_2\text{O}$, 0.0013; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.004; $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.0067; peptone, 0.5 and 40g each of the pretreated cassava peels, Yam peels, Sweet potato peels and Cocoyam peels in their respective flasks. Streptomycin (0.1%) was incorporated into each of the media. The initial pH was adjusted to 5.0 and sterilized.

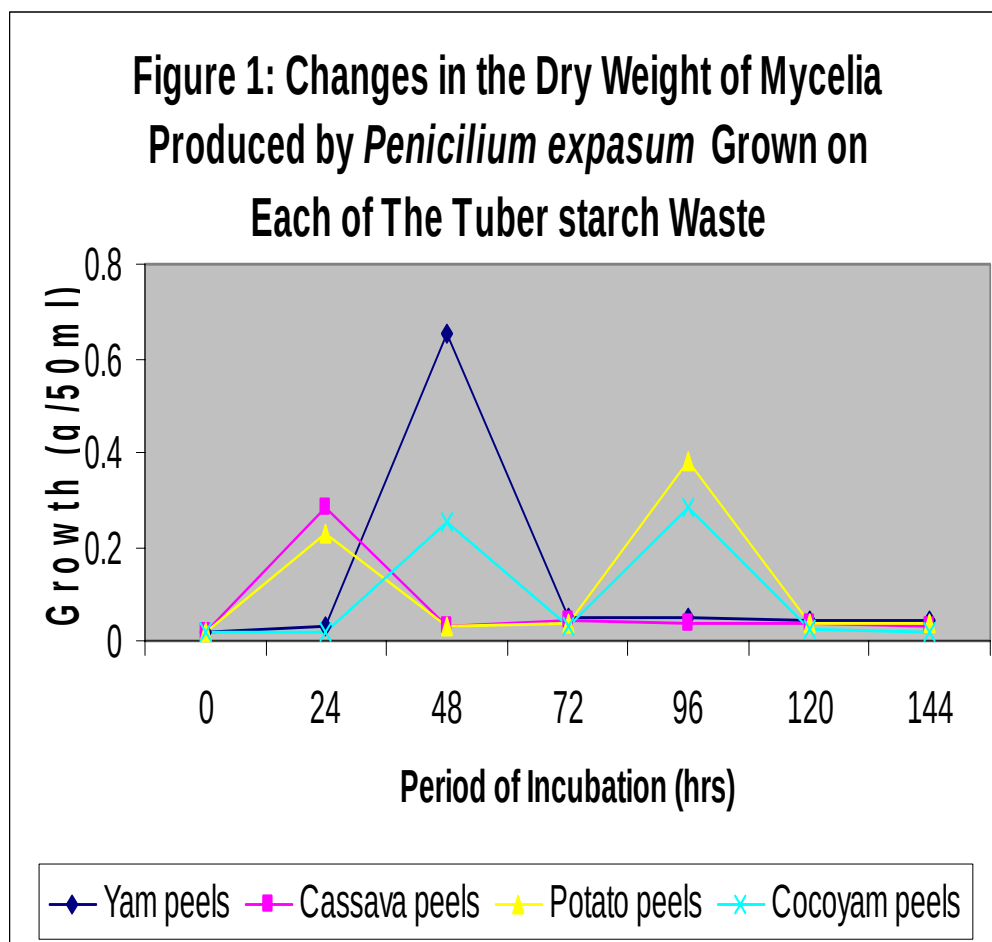
Enzyme Production

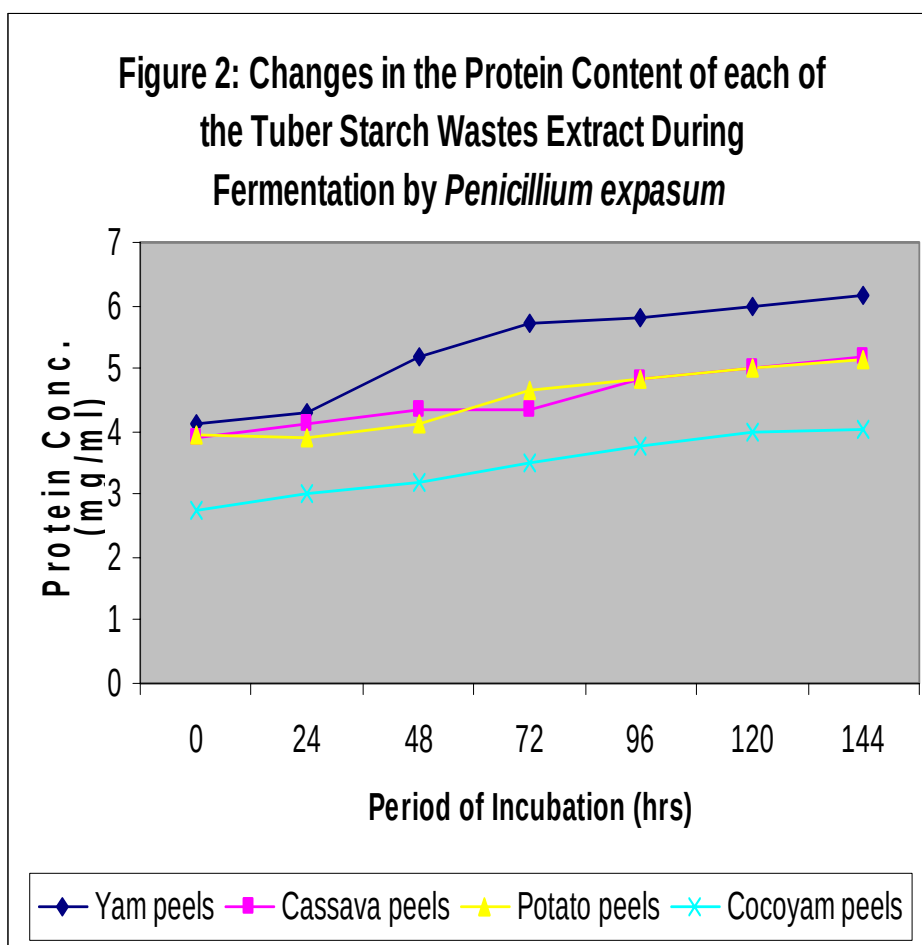
The fungus was grown in the above mineral salt medium for the biodegradation process. A spore suspension (1.0×10^5) per flask of *Penicillium expansum* was used as inoculum for each culture medium. The media were then incubated at 30°C in an orbital shaker (GALLENKAMP) set at 100rpm for 144hours. Six replicates of the fermentation was carried out for each culture medium.

The media were centrifuged at 5,000g for 15mins to obtain crude extract. The method of Dubois *et al.* (1956) was employed in estimating the sugar contents of the culture filtrate. Amylase activity in the extract was also determined using the method of Smith and Roe (1949). Modification of the method was used to determine the residual starch content in the crude extract. The soluble protein contents were determined by the colorimetric method of Lowry *et al.* (1951). Mycelia dry weight method was used for the growth measurement as described by Fawole and Oso (1988).

Results

The ability of *Penicillium expansum* to degrade different tuber starch wastes was studied. The result indicated that the dry weight of mycelia produced by the organism increased progressively from 24 hours of incubation yielding (0.02mg/ml) up to 96 hours of incubation (0.05mg/ml) for the yam peels before the decline. The same pattern was observed for the other tuber peels shown in Fig 1. Soluble protein in each of the extract was increased, with highest yield recorded in yam peel (6.15mg/ml), Cassava (5.2mg/ml), Potato peels (5.13mg/ml) while the least was in Cocoyam (4.05mg/ml) after 144 hours of incubation based on the amount of reducing sugar produced shown in Fig 3. The fungus was able to degrade yam peels (13.0mg/ml) and cassava peels (10.4mg/ml). Sweet potato was least degraded to give (10.2mg/ml) of reducing sugars while cocoyam was partially degraded. The residual starch content was considerably reduced after 144 hours of incubation to give yam peels (6.37mg/ml), cassava peels (5.17mg/ml) and Sweet potato peels (10.03mg/ml). The least was recorded in cocoyam peels (2.0mg/ml) of residual starch shown in Fig 4. Amylase activities in the crude extract is represented in Fig5. The highest amylase activities were detected at 48 hours incubation. The activities decreased thereafter except in the 120 hours of incubation that all tuber starch wastes showed increase except for cocoyam peels.



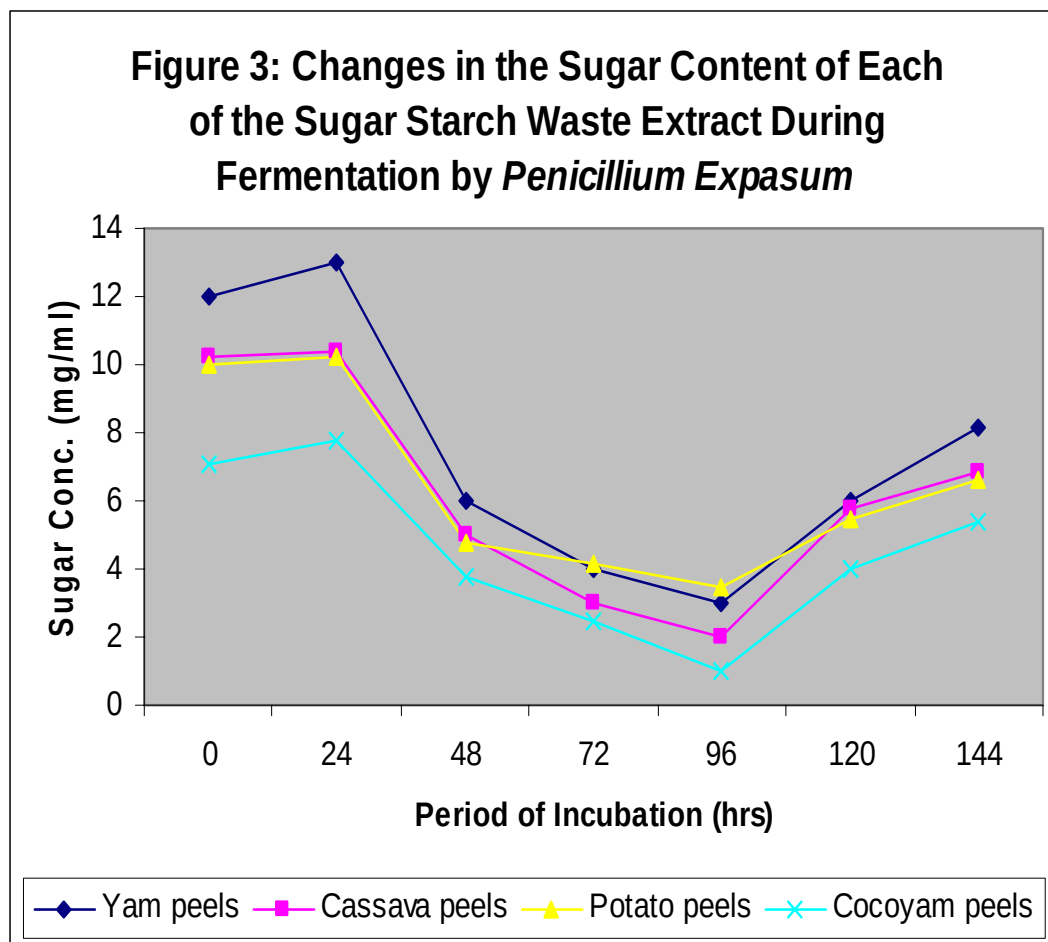


Discussion

The result of this investigation showed that *Penicillium expansum* is able to degrade and cause considerable changes in the nutrient composition of the tuber starch wastes (Cassava peels, Yam peels, Potato peels and Cocoyam peels). This was possibly due to their ability to utilize various organic substances present in the tuber starch wastes as sources of carbon and energy.

The occurrence of amylolytic organisms from the soil agreed with earlier reports of Adebisi and Akinyanju (1998) that the soil is known to be a repository of amylase producer. It was also revealed that Cocoyam peel is less hydrolyzed than potato peels, which was considered as one of the less susceptible to enzyme hydrolysis. The high digestibility of cassava peels observed in this study was similar to that reported by Okolo *et al.* (1995).

Nutritional studies on the fermenting media by various workers have shown that starch is readily converted into sugar by microorganisms, which are able to produce the enzyme amylase; the sugar produced will then serve as the source of carbon and energy for the organism, thereby leading to increase in biomass. Conversely, a decrease in the residual starch could be attributed to the ability of the fungus to hydrolyze starch into glucose and ultimately glucose will be used by the same organism as a carbon source to synthesize fungi biomass rich in protein (Obboh *et al.*, 2002).



The fungus, *Penicillium expansum*, was regulated by the carbohydrate supply. The amylase activity was produced after 48 hours of cultivation which agreed with the result of Nahas *et al.* (2002). The decreased activity in the later phase of growth was probably due to catabolite repression by glucose released from starch hydrolysis as noted by Adams (1985) and Akpan *et al.* (1999b).

The increase in the protein content agreed with the result of Oboh (2006). An increase in the protein content could be as a result of some extracellular enzymes such as amylases, linamarase and cellulose produced by the organisms (Oboh *et al.*, 2003).

The results of this study have shown conclusively the specific roles of *Penicillium expansum* in the biodegradation, fermentation and nutrient utilization of the tuber starch wastes. In deed, the ability of fungi to degrade cellulose has been reported elsewhere (Ofuya and Nwajiuba, 1990; Iyayi and Losel, 2001). Fungi have the ability to produce a variety of enzymes. *Aspergillus niger*, *A. flavus* and *Penicillium* sp, have been reported to be main sources of cellulase, amylase, hemicellulase and xylanase (Hamlyn, 1998). These enzymes help to degrade the non-starch polysaccharide in the substrate to soluble sugar.

Conversion of tuber starch wastes by *Penicillium expansum* means that some of the wastes could be used as raw materials by the starch industry for value added product. This will reduce wastage and improve economic gain. However, whenever it is economically and technologically possible, emphasis should be laid on the utilization of waste materials for recycling and upgrading purposes.

Finally, the problem of tuber wastes disposal will continue to increase in a country like Nigeria with increase in production of tuber products. The only available solution to this problem is to treat some of these accumulated tuber starch wastes with specific microorganisms so as to recycle and if possible convert them to useful materials of high economic value. They could also serve as growth media for fungi and reduce pollution.

Figure 4: Changes in the Residual Starch Content of Each of the Tuber Starch Waste Extract During Fermentation by *Penicillium expasum*

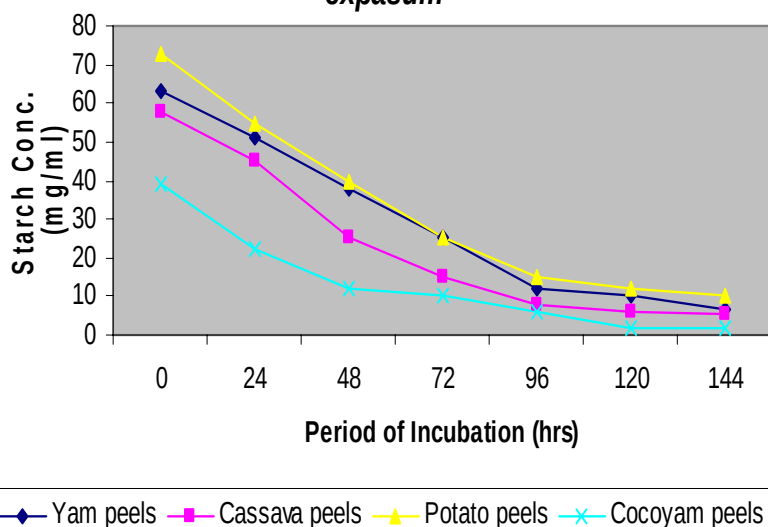
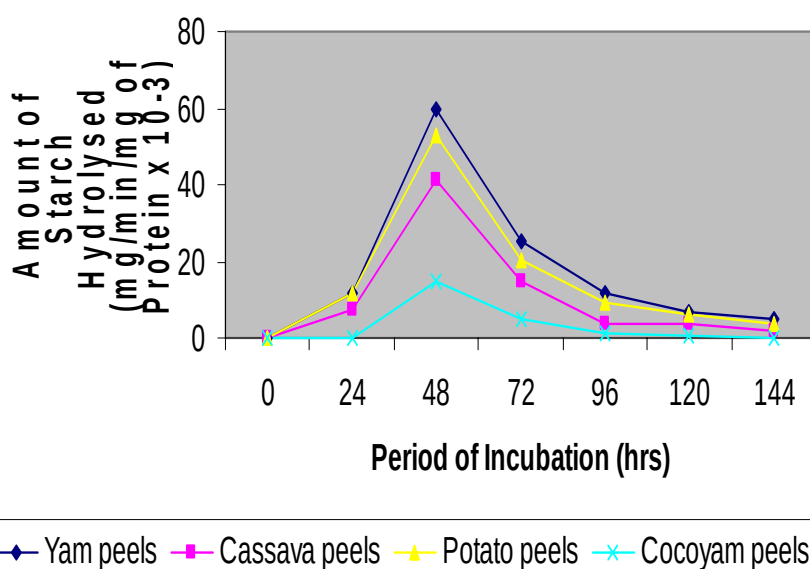


Figure 5: Amylase Activity in the Culture Filtrate of *Penicillium expasum* Grown on Each of the Tuber Starch Waste Extract



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