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Performance of yeast cultures in cassava wastes

Onyegbado C O, Edeh I*

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Abstract

Yeasts isolated from palm wine were cultured in media of varying cassava peel concentration and their growths were monitored. Thereafter, the yeasts were subjected to crude protein analysis. The performance in terms of crude protein and growth rate using monod model was studied. The results show that the maximum crude protein content of about 4% was obtained at 1.5% cassava peel concentration for media unamended with ammonium sulphate. For cassava peel media amended with ammonium sulphate, the maximum crude protein content of about 7% was obtained, also, at 1.5% cassava peel concentration. The maximum specific growth rates were 0.38 day^{-1} for 1%, 0.47 day^{-1} for 1.5% and 0.41 day^{-1} for 2% for amended cassava peel media. For cassava peel media amended with ammonium sulphate, the maximum specific growth rates were 0.9day^{-1} for 1%, 0.94 $day^{-1}$for 1.5% and 0.96 day^{-1} for 2%.

Keywords: Protein enhancer, animal feed, Saccharomyces spp., cassava peel

Introduction

Cassava (Manihot esculenta Crantz) is an essential food crop in the tropics and many countries in Africa. It is cultivated mostly for culinary purposes (Oboh 2006). About 60% of the cassava produced all over the world is used for human consumption (Obadina et al. 2006). Cassava roots play an important role in the African diet and they are processed using simple traditional methods into products such as gari, fufu and lafun flour, some of which are fermented products (Odunfa 1985). In the processing of cassava fermented products, the root is usually peeled to remove the tin brown outer and parenchymatous inner covering, respectively. With hand peeling, the peels can constitute of 20-35% of the total weight of the tuber (Ekundayo 1980).

Onyegbado C O, Edeh I*

Department of Chemical Engineering, University of Port Harcourt, East-West Road, Choba, P.M.B.5323, Rivers State, Nigeria

Email: *ifeanyiexcel@yahoo.com

These peels are usually not good as animal feed, due to their high fibre and low protein contents (Aderolu et al. 2002; Aderemi and Nworgu 2007). Therefore, for cassava peels to be used as a substitute for cereal in animal feeding, they must be supplemented, either with protein-rich oilseed, fish meals or by using microbial techniques. The microbial enrichment process is relatively cheap and the enriched product can increase the potential of cassava as a feed (Balagopalan et al. 1976). One of this microbial enrichment processes involves the use of bacteria or fungi. An example of fungi usually used is yeast. Yeast culture supplements containing *Saccharomyces Cerevisae* which are known to be rich sources of enzymes, vitamins, other nutrients and important co-factors, have been reported to produce a variety of beneficial production responses. These include growth rate, feed intake, feed efficiency, milk composition, egg production and reproduction in ruminants, poultry, pigs and horses.

Some researchers have worked on the enrichment of cassava peel waste using the microbial techniques. However, a crude protein content of about 12.6% using A. niger as the fermenting organism at 35^{o}C has been obtained, although, sugar syrup was added to the fermenting cassava peel medium which brought the moisture content to about 25% (Aderemi and Nworgu 2007). The problem with the addition of sugar is that it is not cost effective in a country like Nigeria, due to near absence of sugar producing facilities. Furthermore, the product of cassava peel fermentation using A. niger has been shown to be toxic to animals when used as feed (Obadina et al. 2006).

This study investigated the performance of yeast culture in cassava waste, such as cassava peels, using yeast isolated from potable palm wine. Fermentor design parameters were also, obtained. No previous work has attempted to obtain these design parameters for cassava peel fermentation.

Materials and Method

Sample collection

The fresh palm wine was obtained from early morning tapings, along the East West Road, while fresh cassava roots were harvested

from a cassava farm located at Ozuoba, both in Obio/Akpor L.G.A. of Rivers State, Nigeria.

Sample preparation

The fresh palm wine was kept in a refrigerator within two hours of collection, to prevent fermentation. The roots of the harvested cassava were washed in order to remove soil particles and were also peeled. The peels were then dried to constant weight and milled. The milled product was stored in a clean plastic container.

An aliquot of the palm wine was diluted to 10^{-2} and cultured on two petri dishes containing SDA acidified with lactic acid. The culture was incubated at 25 – 28°C. After 48 hours, cultures of organisms appeared on the petri dishes. A pure culture of the desired strain was obtained after series of streaks on SDA containing lactic acid. This organism was incubated on acidified SDA slant in a screw cap bottle and stored in a refrigerator. It was then identified based on colour, shape, colony size and mode of reproduction. Also, biochemical tests were carried out in order to identify the organism. These tests include: indole, citrate, catalase, gram reaction tests, and the ability of the organism to ferment sugars such as glucose, sucrose, mannitol, and lactose. The test strain was made to grow on different media prepared in 250ml Erlenmeyer flasks. 1%, 1.5% and 2% (w/v) cassava peels media were prepared by weighing 1g, 1.5g and 2g of dried ground cassava peel into a small quantity of distilled water, stirring and making up to 100ml. Another set of 1%, 1.5% and 2% (w/v) cassava peels media enriched with 1% (w/v) $(NH_4)_2SO_4$ were also made by adding 1g of $(NH_4)_2SO_4$ to the media. The pH of the media was taken. The flasks were stoppered loosely with cotton wool, sterilized by autoclaving for 15 minutes at 121°C and allowed to cool. A loopful of organism from the stock cultures in the previously stored agar slants was dispersed in physiological saline (0.85% w/v NaCl) and diluted to 10^{-2}. 1 ml of the diluted stock was used to inoculate each of the flasks as much as possible. The flasks were left to incubate at room temperature for 10 days.

The Total Viable Count (TVC) was carried out by plating out in duplicates every 48 hours. 1 ml of the culture was diluted and plated in duplicates on petri dishes containing acidified SDA. The petri dishes were incubated at room temperature for 48 hours and the number of colony forming units was manually counted.

Crude protein analysis of the microorganism was carried out using Kjedahl process after 14 days of culture. Fresh organism, 0.1g (aseptically scrapped from the top of the culture), was digested with 15 ml H_2SO_4 and 4g of digestion catalyst (20g Na_2SO_4 and 1g $CuSO_4$ mixed thoroughly) by heating on a hot plate, till a pale green colour persisted. The digest was made up to 100ml. 20ml of the diluted digest was measured into a distillation flask and 20ml of 40% (w/v) NaOH was added to produce an alkaline solution having a dirty brown colour. 10ml of 2% boric acid was measured and poured into a beaker and 2 drops of double indicator was added. The boric acid had a purple colour. The content of the distillation flask was distilled into the beaker containing the boric acid. When a green colour persisted, the distillate was titrated against 0.1M HCl until the colour changed from green to purple. The volume of HCl used was recorded as titre value. This titre value was then used in Equations (2a) and (2b) to calculate the % crude protein.

Basic equations

The number of colony forming units (N) can be calculated using Equation (1).

$$N = \frac{n}{DF \times 0.1} \qquad (1)$$

Where n = number of colony forming units manually counted
DF = dilution factor

$$\%\ Nitrogen = \frac{titre\ value \times 100 \times 100 \times 1.4}{20 \times 1000 \times wt.\ of\ sample} \qquad (2a)$$

$$\%\ Crude\ \mathrm{Pr}otein\ =\ \%\ Nitrogen \times 6.25 \qquad (2b)$$

The kinetics of the growth for the organism media was modeled according to the Equation (3a) and (3b)

$$N = N_0\left[\exp(\mu t)\right] \qquad (3a)$$

which provides an acceptable determination of the specific growth rate, μ

$$\mu = \frac{\Delta \ln N}{\Delta t} \qquad (3b)$$

The maximum specific growth rate was also determined using the Lineweaver-Burk equation (Opara 2002), Equation (4).

$$\frac{1}{\mu} = \frac{k_s}{\mu_m S} + \frac{1}{\mu_m} \qquad (4)$$

Where, μ = specific growth rate (day^{-1}), μ_m = maximum specific growth rate (day^{-1}), S = substrate concentration (wt %) and k_s= saturation constant (wt %)

In plotting the Lineweaver-burk graph, the varying values of the substrate concentration with time were obtained from the relationship (Opara 2002):

$$\gamma\,\partial S\,\partial t = \partial N \partial t \qquad (5)$$

Where γ is the number of the colony forming unit produced per unit concentration of substrate (cassava peel) consumed. Equation 5 was approximated to the total differential form

$$\gamma \frac{\Delta S}{\Delta t} = \frac{\Delta N}{\Delta t} \qquad (6)$$

because of the large values of the time steps (2 days). γ is usually a constant (Opara 2002). Equation 6 can be further simplified as follows:

$$S_i = \frac{S_{i-2} - N_i - N_{i-2}}{\gamma} \qquad (7)$$

Where S_i is the value of the substrate concentration (cassava peel) at the end of the *(i-1)th* time interval N_i the number of the colony forming unit at the end of the *(i-1)th* time interval i = 2, 4, 6,8,10

Results and Discussion

The organism was identified to have the following characteristics: milky white colour, round and serrated edge, moist and raised colonies. Under the microscope, it was found that they showed internal budding and thick ovoid cells singly and in clusters. The results of the biochemical test carried out on the isolated cultures are presented in Table 1. The pH of all the culture media was found to be 7. This is the optimum pH of yeast and it is in agreement with the

pH values of 3 - 8.5 presented by (Adoki 2002). It was also observed that the cassava media gelatinized on autoclaving. This is therefore a clear indication that semi-solid state fermentation was prevalent.

Table 1: Results of the biochemical test for the identification of the organism

TEST	RESULT
Glucose fermentation	Gas and acid production
Sucrose fermentation	Gas and acid production
Mannitol fermentation	Gas production
Lactose fermentation	No reaction
Indole test	Negative
Citrate test	Positive
Catalase test	Positive
Gram reaction	Positive

Considering the above results, the characteristics of the isolated organism showed that it is a yeast-*Saccharomyces spp.*

Figure 1 shows the trends for yeast cell growth over time for an unamended media. It appears to follow monod plot with four phases. From this figure, it can be observed that *Saccharomyces spp.* had progressive growth in all the cassava media. 1.5% media had the highest total viable count (TVC) at 10 days followed by 1% and 2% media. This shows that 1.5% medium is the optimum and yeast cell growth thrives less at a higher concentration of the media.

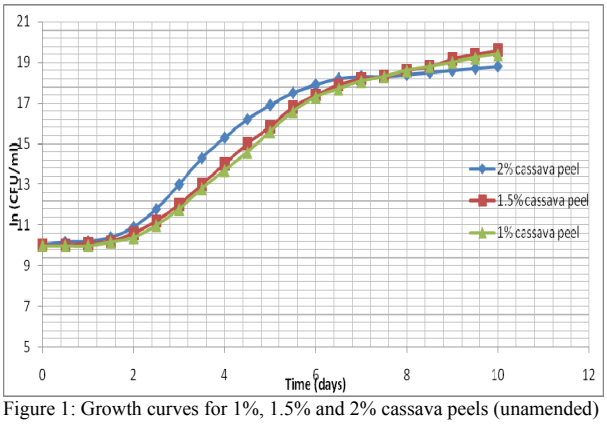

Figure 1: Growth curves for 1%, 1.5% and 2% cassava peels (unamended)

The yeast grown in the 1.5% and 2% cassava media experienced suppressed growth after day 7 in the unamended media. This could be attributed to the suspected catabolite repression, since in an anerobic conditions, yeast produces alcohol which could retard its growth or even lead to the death of such yeast. In Figure 2, the same trend was repeated. Although, due to supplementation with ammonium sulphate, the growth of the cell was more vigorous and there was increase in TVC values, as can be seen from day 8.

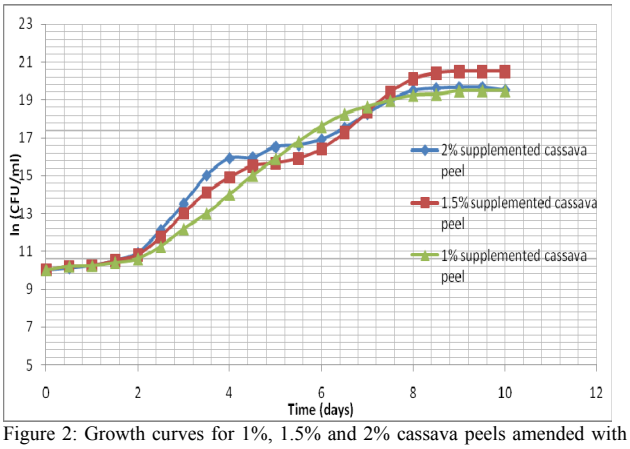

Figure 2: Growth curves for 1%, 1.5% and 2% cassava peels amended with $(NH_4)_2SO_4$

Figures 1 and 2 appear to resemble monod kinetics model, Equation (4). Hence, attempts were made to fit these data with monod equation by plotting the Lineweaver-Burk plot (Figure 3).

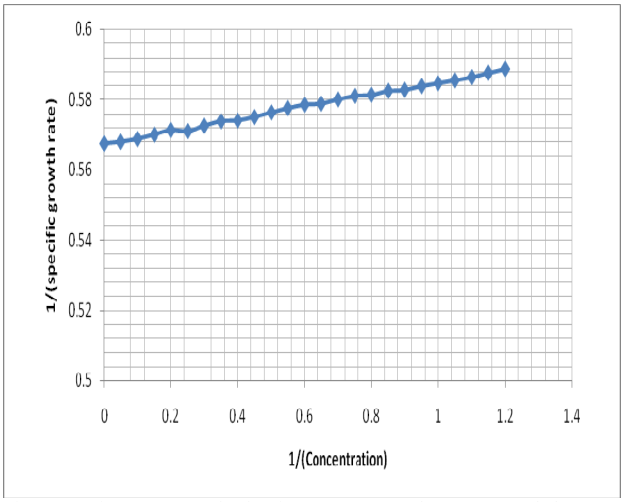

Figure 3: Lineweaver-Burk plot for 1%, 1.5% and 2% unamended cassava peel media

This met with reasonable success. The kinetic parameters (Tables 2 and 3) when the data was fitted to the Lineweaver-Burk plot are presented.

Table 2: Kinetic parameters of cassava peel media unamended with ammonium sulphate

Cassava peel concentration	Maximum specific growth Rate(μ_m) (day^{-1})	Saturation constant (K_s) (wt %)	Coefficient of regression (R^2)
1.00%	0.38	0.008	0.98
1.50%	0.47	0.04	0.82
2.00%	0.42	0.04	0.75

Table 3: Kinetic parameters of cassava peel media amended with ammonium sulphate

Cassava peel concentration	Maximum specific growth Rate(μ_m) (day^{-1})	Saturation constant (K_s) (wt %)	Coefficient of regression (R^2)
1.00%	0.90	0.04	0.95
1.50%	0.94	0.09	0.93
2.00%	0.96	0.08	0.81

A maximum specific growth rate of 0.46 hr^{-1} for pineapple cannery effluent unamended with ammonium sulphate using a yeast, *Candidas utilis* has been obtained (Nigam 1998). This differs from the values obtained in this work for unamended cassava peel medium. The difference may be attributed to the possible higher sugar content of the pineapple waste effluent. Yeasts are able to utilize simple sugars faster than more complex carbohydrates and cellulose used in this work.

Crude protein content of 55.3% on the freeze-dried yeast has also been obtained (Nigam 1998). In the present investigation, the crude protein content (on fresh weight basis) was 2.63%, 3.50% and 3.06% for 1%, 1.5% and 2% cassava peel media respectively, unamended with ammonium sulphate. For media amended with ammonium sulphate, the values of crude protein contents were 3.06%, 7% and 3.94% for 1%, 1.5% and 2% cassava peel media respectively, on a fresh weight basis. The crude protein contents were determined by the Kjedahl method (Ferguson 2000).

The values of crude protein content obtained for 1.5% cassava peel media, were the highest for both amended and unamended media and compared favourably with the crude protein content of human breast milk (4%) and cow milk (8%) on a fresh weight basis. Therefore, the product of the fermentation of the 1.5% cassava peel medium, amended with ammonium sulphate obtained in this work can be concentrated by the same processes that have been used for cow milk to the same level of crude protein content that apply to dried cow milk. The dried product can then be mixed with animal feed for protein enrichment. This makes the fermentation of cassava peels with *Saccharomyces spp.* a good protein enhancer for animal feed.

A value of protein content higher than that obtained in this work has been reported (Obadina et al. 2006). The work used *A. niger*, *A. flavus* and *fumugatus* isolated from cassava wastes. The use of the enriched cassava waste obtained using these organisms are limited, due to the toxic materials produced by them. In this work, yeast isolated from portable palm wine has been cultured to produce single cell protein and the material produced is not expected to be toxic when used as animal feed.

In addition, A. *niger* fermentation in liquid medium has been known to involve the formation of filament and pellets of the micro organism. Oxygen transfer limitations become critical and the pellets have been known to decay from the inside because of the mass transfer limitation of the dissolved oxygen, depending on the flow rate in a continuous fermentation or agitation speed in a batch fermentor (Patel and Thibault 2004). Reactor design becomes difficult and limiting. Continuous fermentation is known to bring about improved constant product control when compared to batch fermentation (Liu and Zajic 1973). Attempts have been made to ameliorate this problem by using the loop fermentor. Some researchers have carried out batch fermentation in Erlenmeyer flask (Aderemi and Nworgu 2007), without any consideration to fermentation design kinetic parameters which could aid the large scale design and development of the fermentation system. Sugar supplementation is also not acceptable where there is scarcity of the sugar as it is evident in a developing country like Nigeria, without sugar production facility. In this study, attempts have been made to utilize a completely waste material for the production of animal feed and also help in environmental management.

The fermentor design from this study is realizable because a dilute system has been employed. A micro organism regularly used in the brewing industries has also been employed. Therefore, continuous or batch system that use yeast cells are potentially applicable. Another important advantage of this work is that it was done at room temperature. This obviates the need for refrigeration usually encountered in the alcoholic fermentation using yeast cells (*Saccharomyces cerevisae*). This will further lead to the cost saving in the production of the crude protein using cassava peels.

Mixed cultures have also been found to increase the crude protein content and reduce the levels of toxic substances like cyanide and phytate of cassava peels after fermentation (Oboh 2006).

Conclusion

This work shows that fermentation enhances crude protein content of cassava peels. The crude protein content obtained for 1.5% cassava medium supplemented with ammonium sulphate compares favourably well with that of cow milk on a fresh weight basis. It can therefore be concentrated to the same levels as the dried cow milk. This makes it a good protein enhancer for animal feed. Previous works using *A. niger*, even though, they led to higher protein concentration are inapplicable because they lead to production of materials which are toxic to animals and also add sugar to the fermentation broth. This work uses a completely waste material and leads to better environmental control. In addition, the use of protein enriched cassava peels in animal feed will save maize for other uses, including gashol production. Fermentor design using results of this work should prove easier than the work using *A. niger* because a dilute system has been used.

Finally, the kinetic parameters obtained in this work: the specific growth rate, the optimum substrate concentration level, and the saturation constant will aid the design of fermentors for the protein enrichment of the cassava peel mash. This is the only known attempt at obtaining such design parameters for the cassava peels fermentation.

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Nomenclature

$\Delta \ln N$ change in ln N
Δt change in time (days)
μ specific growth rate (day^{-1})
μ_{max} maximum specific growth rate (day^{-1})
k_s saturation constant (% w)
n number of colony forming units manually counted
DF dilution factor
S substrate concentration (wt%)
γ the number of the colony forming
S_i the value of the substrate unit produced per unit concentration (cassava peel) of substrate concentration (cassava peel at the end of the (i-1)*th* time interval
N Colony forming unit (cfu/m)
N the number of the colony forming unit at the end of the (i-1)th time interval