Isolation, culture characteristics and factors affecting growth and biomass production of a strain of *Candida* sp. used for single-cell-protein-enriched feed supplement production

AKURO ADOKI & ABIYE ADOKI

(Akuro Adoki: Institute of Pollution Studies, Rivers State University of Science and Technology, P.M.B. 5080, Nkpolu - Oroworakwo, Port Harcourt, Nigeria; Abiye Adoki: Department of Geography, Rivers State College of Agricultural Science, P.M.B. 5936, Port Harcourt, Nigeria)

SUMMARY

During the preliminary phase of a process for the production of a protein-enriched feed supplement, a strain of *Candida* sp. was isolated from ripe banana pulp. Morphological and biochemical tests showed that the strain which was bipolar and elongated was not capable of growth at 37 °C but grew only on dextrose and fructose and was able to supply its amino acid requirements in culture. The efficiency of conversion of readily utilizable carbon sources in fruit wastes used for feed supplement production by the test strain was studied. Results obtained showed that reducing sugars were most readily utilized compared to other soluble carbohydrates. Conversion efficiencies were high and averaged about 75 per cent in unsupplemented substrates. Supplementation with organic and inorganic compounds resulted in greatly increased biomass yields.

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RÉSUMÉ

ADOKI AKURO & ADOKI ABIYE.: *L'isolement, les caractéristiques de culture et les facteurs influençant sur la croissance et la production de biomasse d'une lignée de* Candida *sp. utilisée pour la production d'un régime complémentaire unicellulaire-protéine-enrichie.* Au cours de la phase préliminaire d'un procédé pour la production d'un régime complémentaire enrichi-de-protéine, une lignée de *Candida* sp. était isolée de la pulpe de banane mûre. Les tests morphologique et biochimique démontraient que la lignée qui était bipolaire et allongée n'était pas capable de croissance à 37 °C mais croissait au dextrose et au fructose et était en mesure de fournir ses exigences d'acide aminé en culture. L'efficacité de la conversion des sources de carbone aisément utilisable dans les déchets de fruits utilisés pour la production de régime complémentaire par le test de lignée étaient étudiés. Les résultats obtenus montraient que les sucres réduisants étaient les plus aisément utilisés en comparaison des autres féculent solubles. Les efficacités de conversion étaient élevées et faisaient environ 75 pour cent moyenne dans les substrates non supplémenté. La supplémentation avec les composés organiques et inorganiques aboutissait à des rendements de biomasse largement augmentés.

Introduction

Recently, there has been considerable emphasis on the world food crisis and on the availability of "waste" products which could be used to alleviate food shortages (Bhattacharjee, 1970). Many of the developing countries with major nutritional problems produce an excess of materials rich in carbohydrates that can be used in fermentation processes to produce microbial protein which can be used in turn to upgrade both human and animal feeds. Traditional protein sources are relatively more expensive and the absence of well-developed technological facilities has contributed to losses incurred through spoilage of even the limited avail-

able sources. Consequently, alternative ways of meeting the protein demands must be explored. Compared to the developed countries, the less-developed countries appear to have abundant supply of agro-waste substrates that could be converted to additional protein sources (Moo-Young, 1977).

The aim of this study was, therefore, to show the use of fruit wastes to produce microbial protein that could serve to supplement the available traditional protein sources. An attempt was accordingly made to produce single-cell-protein-enriched (SCP) feed supplement from a *Candida* sp., using wastes derived from orange (*Citrus sinensis*), banana (*Musa paradisiaca*), and plantain (*Musa sapientum*). These raw material sources were chosen as substrates because wastes derived from them, for example, as obtained in the processing of orange for juice or jam production, are enormous and frequently create environmental problems associated with non-effective disposal systems. Similarly, a lot of wastes are produced from these fruits as a result of spoilage of harvested fruits due to inadequate storage facilities. The magnitude of such spoilage could be well appreciated when local markets are surveyed. The microbe, *Candida* sp., was chosen because of its relatively very good isolation and growth on carbohydrate-containing media tested. Similarly, its energy requirements were considered to be minimal as it grew very well at room temperature.

Materials and methods

Isolation

The yeast strain used for the study was isolated from ripe banana. Fifty grams of fresh ripe banana pulp was marshed in a pre-sterilized mortar, and transferred aseptically to 100-ml sterile distilled water in a 250-ml conical flask loosely plugged with a cotton wool. This was left to stand for 48 h and a loopful cultured on antibiotic-containing potato dextrose agar (PDA) described by Cruickshank *et al.* (1975) at 25 °C for 24 h. Stock cultures of the isolated strain were maintained on agar slants of antibiotic-containing PDA in screw-capped bottles and stored at 4 °C.

Morphological characterization of test strain

The morphological characteristics of the isolate were determined by microscopic examination of the colony size, shape, colour, and cellular morphology.

Biochemical characterization of test strain

As an aid to further identification of the production strain, some biochemical tests were carried out as follows:

1. *Utilization of carbon and energy sources.* Biochemical tests involving the utilization of dextrose, fructose, maltose, mannitol, lactose, and sucrose were carried out as described by Cruickshank *et al.* (1975) and Bradshaw (1979).

2. *Growth at 37 °C.* This was tested by streaking duplicate PDA plates with yeast cells from a slant culture and incubating the plates at 37 °C for 24 h, after which the plates were observed for growth by the development of visible colonies.

3. *Amino acid requirements.* The modified minimal medium as outlined by Cruickshank *et al.* (1975) was used to determine the amino acid requirements of the test strain. One litre of the complete medium contained the following supplements: adenine (20 mg), arginine (20 mg), histidine (20 mg), isoleucine (20 mg), leucine (20 mg), lysine (20 mg), methionine (20 mg), phenylalanine (40 mg), threonine (200 mg), tryptophan (30 mg), tyrosine (20 mg), uracil (20 mg), and valine (20 mg). Selective omission media were prepared by omitting individual amino acids or bases from the complete medium. This allowed the identification of the growth requirements of the test strain (Hawthorne & Mortimer, 1960).

4. *Enzyme assay.* The saccharolytic activity of the test strain was tested by growing the organism on starch agar (Bradshaw, 1979); and regarding cellulolytic activity, by adding an aliquot of a culture filtrate of the organism to a solution (suspension) of cellulose in 0.55 M acetate or 0.055 M citrate buffer (*p*H 5.5) and incubating the resulting enzyme-substrate solution at room temperature for

about 10 days (Reese & Mandels, 1963).

5. *Shake flask cultures.* Studies of the test yeast strain as regards its growth characteristics in fruit waste media were carried out in shake flasks.

Basal media

Dry, ground orange, plantain, and banana wastes were used to prepare the basal media for these studies. Each basal medium was prepared by suspending 2.0 g of dry, ground wastes in 100-ml deionized water and *p*H adjusted to 4.6 with citrate-phosphate buffer as described by Cruickshank *et al.* (1975). The fruit waste suspensions were then autoclaved at 121°C for 15 min and allowed to cool.

Inoculation of flasks

Flasks were inoculated after coolings with 1 ml portions of a suspension of yeast cells in sterile distilled water. These were then incubated in an orbital shaker set at 150 rpm at room temperature (25-28°C).

Determination of sugar utilization (conversion) efficiency

Only unsupplemented media were used to investigate the efficiency of conversion of reducing sugars and other soluble carbohydrates by the test strain. Allen *et al.* (1974) and Oso (1978) showed that the efficiency of conversion can be derived thus:

$$\text{Conversion efficiency (\%)} = \frac{\text{Initial concentration of carbon source} - \text{Lowest concentration recorded}}{\text{Initial concentration}} \times 100/1$$

The level of reducing sugars at different intervals of the incubation period was determined by aseptically withdrawing sample volumes (2 ml) of the culture suspension into sets of clean centrifuge tubes and centrifuging at the high speed setting using MSE model Minor 35 centrifuge to obtain clear supernatants. One millilitre supernatant portions were then pipetted into clean test tubes and the amount of reducing sugars determined by the dinitrosalicyclic acid method described by Oso (1978).

The levels of total soluble carbohydrates were similarly determined on separate 1 ml supernatant volumes. The method used was the anthrone procedure described by Allen *et al.* (1974).

Yeast cell biomass production

1. *Effect of substrate supplementation on biomass yields.* For this determination, only supplemented dry, ground orange wastes were used with unsupplemented media as controls. The media suspensions previously described were supplemented with 0.025 - 0.20 per cent w/v dextrose and 0.1 - 0.8 per cent w/v ammonium nitrate (NH_4NO_3) in duplicates. These were sterilized and inoculated as described previously. Inoculated flasks were incubated at room temperature on an orbital shaker for 60 h. The solid fractions of the duplicate uninoculated flasks were harvested by centrifugation and supernatants decanted off. The harvested solids were then washed three times with sterile distilled water with centrifugation carried out intermittently between washings. The washed solids were then dried in a hot-air oven at 80 °C until constant weights were obtained. The difference between the weights of the uninoculated flasks represented biomass yields.

2. *Comparison of biomass yields in different substrates.* Biomass production in supplemented and unsupplemented orange, plantain , and banana waste media were compared. The preparation of the media and further steps in this determination were as previously described.

Results and discussion

Growth of the organism on potato dextrose agar (PDA) showed that it developed creamy-white, entire-raised colonies which were 1-2 mm in diameter after 24 h. Microscopic examination showed that the cells were gram-positive, elongated and multiplied by bipolar budding (Table 1). Fermentation tests with the test strain yielded positive results with dextrose and fructose but negative for maltose, sucrose, mannitol, and lactose (Table 1).

Negative tests were indicated by the absence of acid or gas production in the fermentation broth.

Enzymatic studies for the production of amylases and cellulases showed that the test strain was unable to use starch and cellulose as sources of carbon and energy. Further physiological tests showed that the test strain produced discrete colonies on PDA on incubation at room temperature but failed to grow at 37 °C. Since one of the requirements of human microbial pathogens is the capability to grow at 37 °C, this result presumably indicates the safety of the use of this organism for animal feed supplement production.

TABLE 1

*Morphological and Biochemical Characteristics of the Test Strain (*Candida *sp.)*

Morphological characteristics		*Biochemical characteristics*	
Colony morphology	entire-raised	Dextrose	+
Colony colour	creamy-white	Fructose	+
Budding pattern	bipolar	Maltose	-
Cell shape	elongated	Mannitol	-
Staining reaction	gram-positive	Lactose	-
Growth at 37 °C	-	Sucrose	-
		Starch	-
		Cellulose	-

An investigation of the amino acid requirements of this strain showed that it was capable of growth on selective omission media lacking the amino acids screened (Table 2). This observation agrees with earlier studies by Snyder (1970), Dimmling & Seipenbusch (1978), Labaneiah *et al.* (1979), and Okada, Ohta & Ebine (1980). The test strain was therefore capable of synthesizing the amino acids and bases investigated, and could be cultivated and harvested as a nutritional source of these amino acids in feed supplement production.

TABLE 2

*Amino Acid Requirements of Test Strain (*Candida *sp.)*

Amino acid/base deficiency	*Result (growth)*
Tryptophan	+
Methionine	+
Isoleucine	+
Arginine	+
Histidine	+
Threonine	+
Adenine	+
Uracil	+
Phenylalanine	+
Tyrosine	+
Leucine	+
Lysine	+
Valine	+

Fermentation/substrate utilization studies

Comparison of growth in fruit wastes. A comparative study of the growth levels of the production strain (Fig. 1) in unbuffered suspensions of the fruit wastes showed that highest growth, as log total viable count, was obtained for the orange waste (11.69), with slightly lower value for plantain (10.34) and much lower value for banana peels (8.5) at 33 h after incubation.

The growth levels in the three substrates were compared using mixed substrate sources (Fig. 2). Results show that the growth level was highest (12.5) for the orange-plantain combination and slightly lower (11.7) for the plantain-banana combination. The growth level for the orange-banana mixture was about two log cycles lower than the previous ones. The results also showed that growth was optimal at about 33 h, followed by a sharp decline up to 66 h, and then rose again. This suggested the use of more than one substrate as source of carbon and energy in the medium. The 33-h growth period contrasts with the 60 h recorded when the medium was buffered at *p*H 4.0-5.0 (Fig. 3). Regarding economics and time, the unbuffered could be assumed to be better, as this fermentation option would provide for savings on energy costs, manpower hours, and rate of production, i.e. turnover (Trilli, 1977; Moo-Young, MacDonald & Ling, 1981; Gonzalez-Valdes & Moo-Young, 1981).

Determination of optimum p*H for growth.* During growth in culture media one of the factors

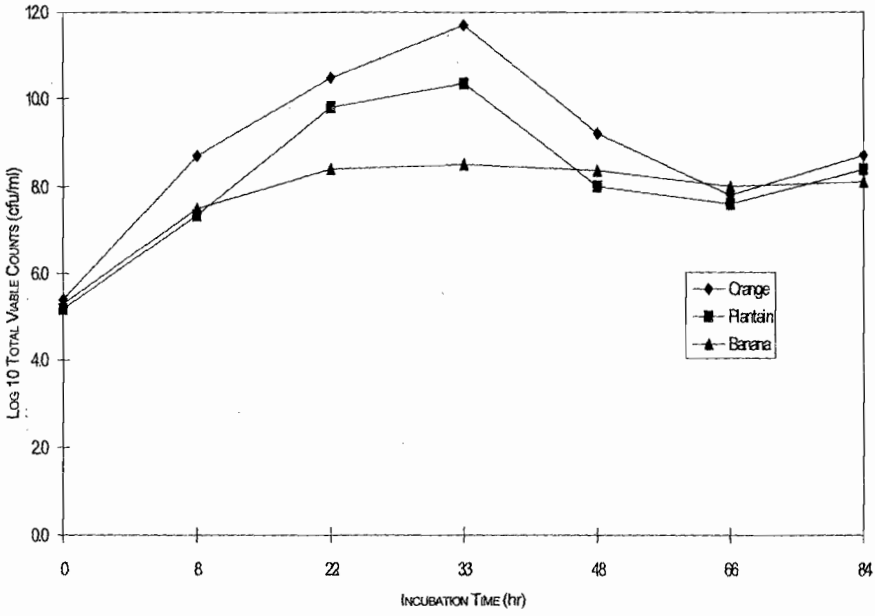

Fig. 1. The growth of *Candida* sp. in unsupplemented substrates of orange, plantain and banana (*p*H unadjusted; 25 °C).

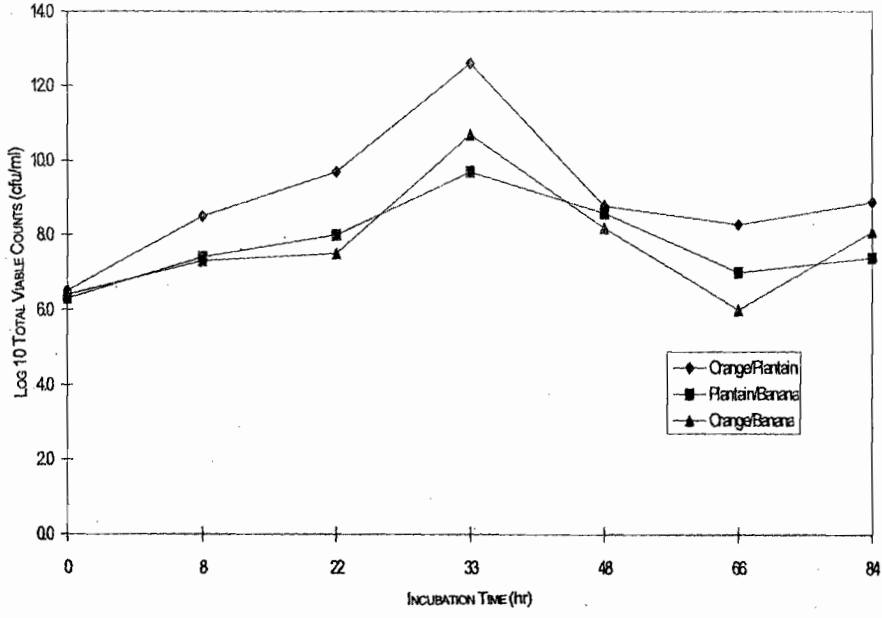

Fig. 2. The growth of *Candida* sp. in unsupplemented mixed substrates of orange/plantain, plantain/banana and orange/banana (*p*H unadjusted; 25 °C).

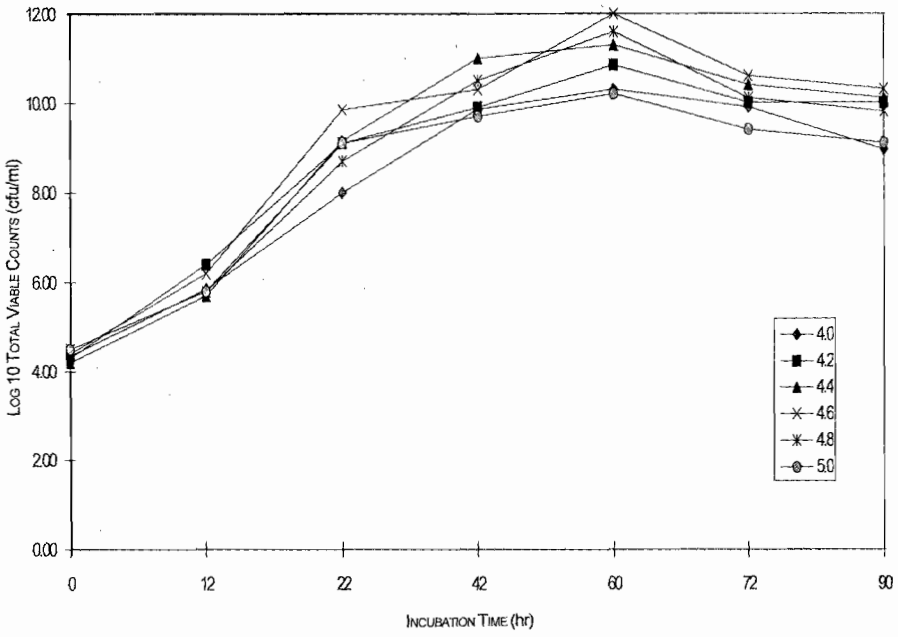

Fig. 3. Determination of optimum *p*H (4.0-5.0) for growth of *Candida* sp. on unsupplemented orange waste at 25 °C.

controlling the level of growth of any selected strain is the *p*H (Cruickshank *et al.*, 1975). The *p*H would also determine the occurrence and types of particular contaminants in the culture medium. It is, therefore, necessary that the *p*H of growth of any selected strain is optimal for this strain and at the same time reduces the growth of contaminants. Preliminary investigations showed that the test strain was capable of growth over a wide *p*H range of 3.0 to 6.2 (data not presented). The results also showed that favourable growth(s) could occur from *p*H 3.0, reaching an optimum at *p*H 4.6 (Fig. 3). For further studies, the *p*H value of 4.6 was therefore selected. Below a *p*H level of 4.6 (3.0 and 3.4) and above (5.8), mixing by totary shaking was difficult as the media tended to be viscous.

Since most saprophytic bacteria have wide *p*H growth range and can thrive at *p*H as low as 4.4 (Cruickshank *et al.*, 1975), the optimum *p*H of 4.6 selected for the test strain therefore has limited or reduced selective advantage. Lower growth *p*H within the range studied could be considered for large-scale cultivation of the test strain, as contamination may be more difficult to control at this level. The problem of controlling the observed increase in viscosity of media at these low pH values could be overcome by reducing the incubation period to 33 h.

Determination of optimum substrate concentration. Results of tests with the production strain for determination of the optimum substrate concentration for growth (Fig. 4) showed that growth, as total viable count, was highest (12.1) for the substrate concentration of 2.0 per cent w/v. On the other hand, the growth levels were about equal at substrate concentrations of 1.2 and 1.5 per cent (11.8) and least at 1.0 per cent. The results also show that for the substrate concentrations 1.2, 1.5, 1.7 and 2.0 per cent w/v, the peak growth was obtained after 60 h. Comparatively, for the 1.0 per cent concentration, peak growth was observed after 40 h. A probable reason for this observation is that at 1.0 per cent concentration, the problem of viscosity is not existent and growth proceeds at a

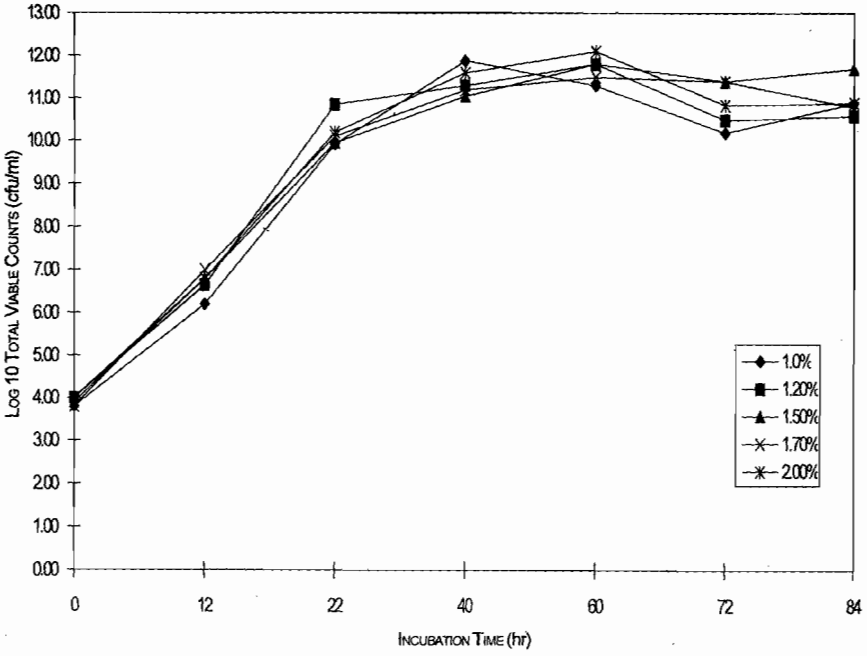

Fig. 4. Effect of substrate concentration on growth of *Candida* sp. on unsupplemented orange waste (*p*H 4.6; 25 °C).

faster rate. However, since the total viable count is paramount in the utilization of the test strain, the attainment of peak growth has to be compromised with increased production time.

Although growth was best at substrate concentration of 2.0 per cent w/v after 60 h when only one growth cycle is considered, the effect of this relatively higher growth is lost after two log cycles when 1.5 per cent w/v concentration is considered at 84 h. What is implied is that the optimum concentration may not necessarily be translated as optimum in economic terms. As was observed during experiments to determine optimum *p*H for cultivation of the test strain, an increase in viscosity of the culture medium was noticed as the substrate concentration was progressively increased. The substrate concentration, and therefore medium viscosity, would influence the growth of the test strain in terms of agitation/aeration efficiency. Solomons (1983) has also reported that substrate concentration affects the yield of *Saccharomyces cerevisiae* when grown on an assimilable carbohydrate such as glucose or sucrose. For this organism he reported that substrate levels in batch culture must be kept very low, otherwise the substrate is oxidised to carbon dioxide and heat. He has similarly stated that *Candida* species are preferred to single-cell-protein (SCP) production because they have better regulation between anabolic and catabolic pathways which prevents waste of substrates. This advantage could have contributed in part to the high yield recorded for the test strain.

Influence of supplementation with carbon and nitrogen on growth of the test strain

Preliminary investigations with the test strain (Adeki, 1987) showed that this organism grew best in culture media supplemented with ammonium nitrate in contrast to supplementation with ammonium sulphate. Addition of phosphorus (KH_2PO_4) to media did not significantly improve growth.

Further tests on the effects of mixed substrate supplementation on growth of the test strain were also carried out (Fig. 5). The highest growth was

recorded at the 0.15/0.6 per cent w/v dextrose/ ammonium nitrate combination, which was more than one log cycle greater than the unsupplemented medium at peak growth point. On the other hand, the lowest growth was obtained with the 0.05/0.2 per cent w/v combination. In all cases, optimal growth was recorded after 60 h incubation at room temperature and *p*H of 4.6.

One criterion that is crucial in the selection of a yeast strain for protein production is the ability to grow in unsupplemented substrates or where supplementation is made it is simple and cheap. This criterion is satisfied by the results obtained where the test strain was found to grow well even in unsupplemented substrates with the exception of banana wastes. The higher level of added nitrogen indicates that the level of nitrogen in the growth substrates was more limiting than that of the sugars. Whilst supplementation with nitrogen source resulted in an increase in growth of the test strain, no such marked effect was observed when the production medium was supplemented with a source of phosphorus. This result could be explained in part by the relatively high levels of phosphorus in the substrates and, compared to carbon and nitrogen, the level of phosphorus required by growing organisms is lower. The 0.2 to 0.4 per cent recorded for the raw substrates (Adoki, 1987) also compares with that obtained for tropical citrus processing by-products by Crandall & Kesterson (1980).

Substrate conversion efficiency

Fig. 6 shows the results of investigations on the test strain substrate conversion efficiency for feed supplement production.

At zero incubation time, the concentrations of total soluble carbohydrates and reducing sugars in the fruit waste suspension growth medium were 6.5 and 3.7 mg/ml, respectively. While there was no decrease in the level of soluble carbohydrates after an incubation time of 12 h, a sharp decline in the

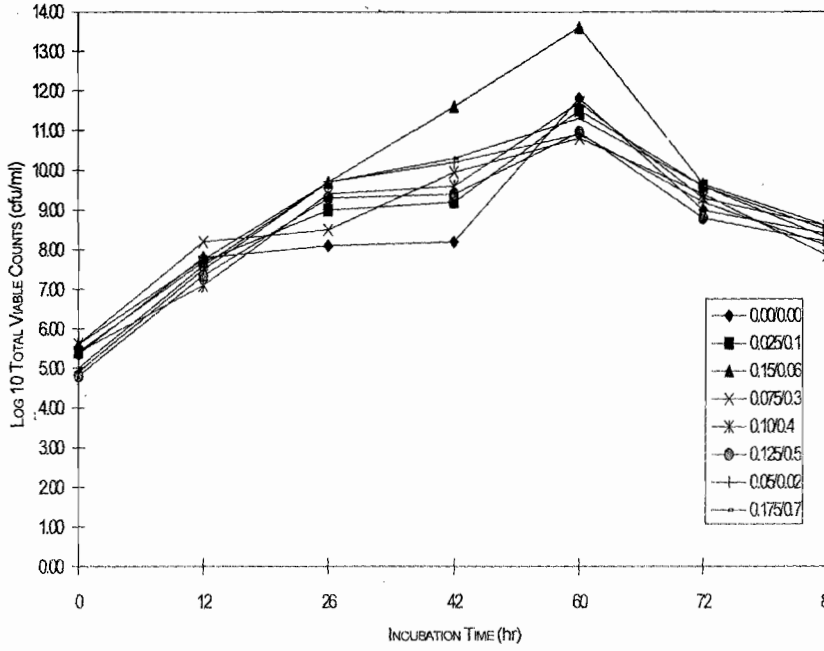

Fig. 5. Effect of supplementation with different dextrose/NH_4NO_3 combinations on growth of *Candida* sp. on orange waste substrate.

level of reducing sugars was noticed for the same period. This indicated that the reducing sugars were more accessible to the test strain as immediate sources of carbon and energy compared to the other soluble carbohydrates. Based on the highest and lowest concentrations of soluble carbohydrates recorded during the incubation period, a utilization efficiency of 67.7 per cent was obtained as compared to 84 per cent obtained for reducing sugars. This indicated greater utilization of the reducing sugars by the test strain.

Fig. 6 also shows the relationship between substrate utilization and cell growth. During the incubation period, cell numbers reflected the sugar utilization patterns. Generally, a decrease in numbers of viable cells was recorded after 36 h, during which period cell death was greater than cell growth. The substrate utilization efficiencies of 67.7 and 84 per cent of the test strain reported earlier were found to be higher than the values reported by Kamel (1979) for four yeast strains and indicate its potential for microbial biomass production.

Yeast cell biomass production

Yeast cell biomass is determined by the level of growth of the test strain which in turn is ascertained by log total viable counts. Fig. 7 shows the effects of the substrate supplementation on biomass yields. Yeast cell biomass production by the test strain was highest at the 0.15/0.6 per cent (w/v) dextrose/ammonium nitrate supplementation level, giving a yield of about 2.03 kg/kg of supplemented orange waste. Comparatively, the yield for the unsupplemented substrate was 0.87 kg/kg of orange waste. At higher supplementation levels, microbial biomass production was found to be lower than that recorded at the 0.15/0.6 per cent w/v supplementation level. The high yield recorded was similar to that reported by Okada, Ohta & Ebine (1980) for *Saccharomyces cerevisiae* based also on the 0.15/0.6 per cent w/v supplementation level. Results with unsupplemented substrates were also similar to those reported by Moo-Young (1977) whose data gave a yield of 0.85 kg/kg substrate.

A comparison of biomass production in orange,

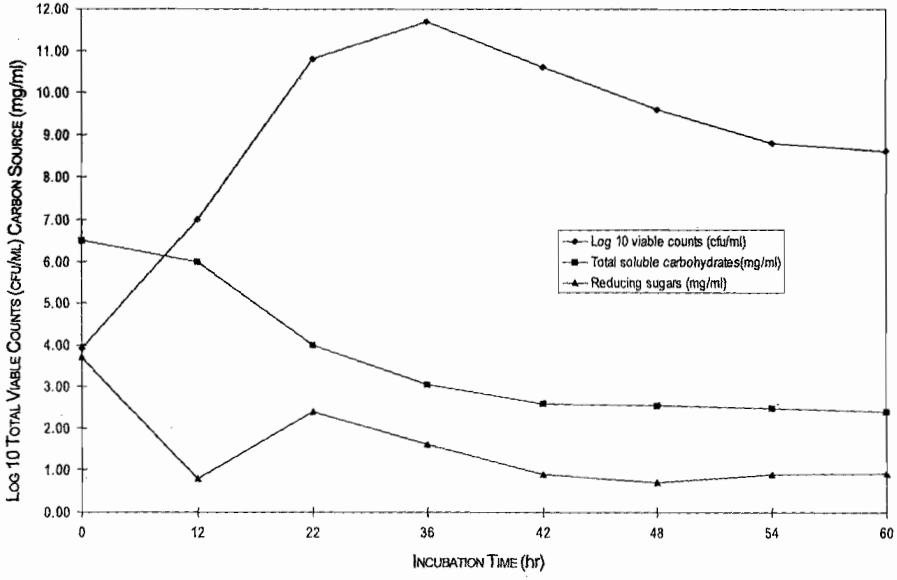

Fig. 6. Utilization of two sources of carbohydrate carbon in orange waste suspension by *Candida* sp. for growth.

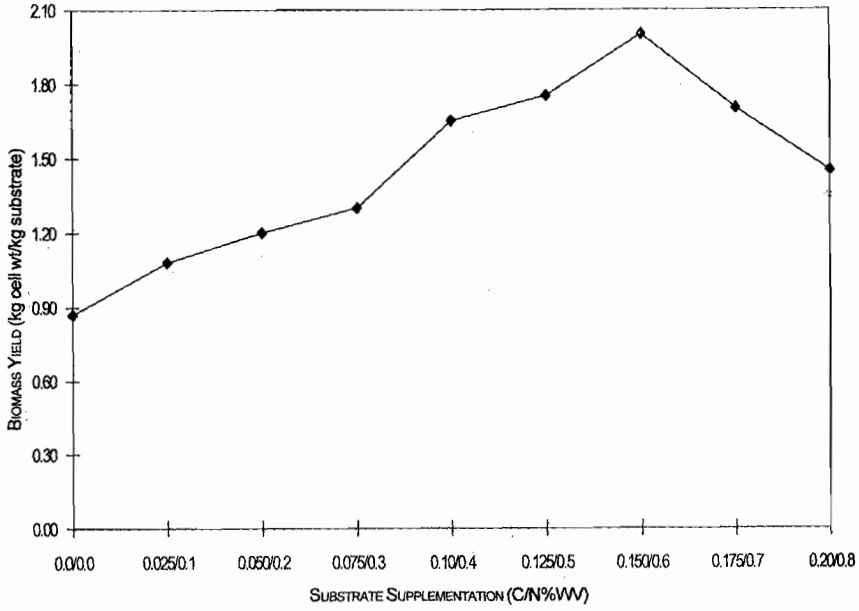

Fig. 7. Effect of supplementation with dextrose and ammonium nitrate on biomass production of *Candida* sp. on orange waste substrate.

banana, and plantain waste media shows that production by the test strain was best in orange media under unsupplemented and supplemented conditions (Table 3). Crude protein contents were also increased by about 100 per cent in orange waste as compared to lower levels recorded for plantain and banana wastes. The level recorded for orange waste is comparable to that reported by Lequerica & Lafuente (1977).

TABLE 3

Comparison of Biomass/Crude Protein Production by Candida *sp. in Unsupplemented and Supplemented Fruit Wastes after 60 h Incubation (pH unadjusted)*

Substrate	*Biomass production (kg cell, wt/kg fruit waste)*		*Crude protein content (percent)*	
	Unsupplemented	*Supplemented*	*Initial*	*Final*
Orange	1.33 ± 0.1	1.60 ± 0.05	8.8 ± 0.3	17.5 ± 0.1
Plantain	0.67 ± 0.2	1.33 ± 0.1	5.4 ± 0.24	8.5 ± 0.15
Banana	0.67 ± 0.1	0.67 ± 0.1	5.1 ± 0.08	7.9 ± 0.01

Conclusion

Investigations were carried out during the study to primarily determine the suitability of the chosen waste raw materials as substrates for the production of protein-rich feed supplements. The findings that the test strain was capable of growth on reducing sugars and some soluble carbohydrates imply that it could be used to reduce the levels of these carbon and energy sources in processing waste streams rich in them. Linked with the high biomass and protein yields recorded during this study, are the high substrate utilization or conversion efficiency and growth rates of the test strain. It could therefore be concluded that the process studied could be exploited commercially to produce protein-rich feed supplements.

REFERENCES

Adoki, A. (1987) *Utilization of fruit waste for production*

of protein-rich feed supplements through microbial fermentation using Candida *sp.* (MSc Thesis). University of Port Harcourt, Nigeria.

Allen, H., Stewart, E., Max Grimshaw, J. A., Parkinson, A. & Quarmby, C. (1974) *Chemical analysis of ecological materials.* Oxford: Blackwell Scientific Publications.

Bhattacharjee, J. K. (1970) Microorganisms as potential sources of food. *Adv. appl. Microbiol.* **13** (139).

Bradshaw, L. J. (1979). *Laboratory microbiology,* 3rd ed. Philadelphia, London: W. P. Saunders Company.

Crandall, P. G. & Kesterson, J. (1980) BOD and COD determinations on citrus waste streams and component parts. *J. Fd Sci.* **45** (134).

Cruickshank, R., Duguid, J. P., Marmion, B. P. & Swain, R. H. A. (1975) *Medical microbiology: The practice of medical microbiology,* Vol. II. London: Churchill Livingstone.

Dimmling, W. & Seipenbusch, R. (1978) Raw materials for the production of SCP. *Process Biochem.* **13** (3), 9 - 15.

Hawthorne, D. C. & Mortimer, R. (1960) Chromosome mapping in *Saccharomyces* centromere linked genes. *Genetics* **45**, 1085 - 1110.

Gonzalez-Valdes, A. & Moo-Young, M. (1981) Production of yeast SCP from corn stover carbohydrates. *Biotech. Lett.* **3** (3), 143 - 148.

Kamel, B. S. (1979) Utilization of date carbohydrate as substrate in microbial fermentation. *Process Biochem.* **14** (16) 12 - 14.

Labaneiah, M. E. O., Abou-Donia, S. A., Mohammed, M. S. & El-Zarki, E. M. (1979) Utilization of citrus wastes for the production of fungal protein. *J. Fd Tech.* **14** (1), 95 - 100.

Lequerica, J. L. & Lafuente, B. (1977) Citrus by-products utilization. II. Semisolid fermentation of orange peels by *Candida utilis*. *Rev. Agroquim. Technol. Aliment.* **12**, 71.

Moo-Young, M. (1977) Economics of SCP production. *Process Biochem.* **12** (4), 6 - 10.

Moo-Young, M., MacDonald, D. G. & Ling, A. (1981) *Biotech. Lett.* **3** (3), 149 - 152.

Okada, N., Ohta, T. & Ebine, H. (1980) Selection of yeast strain and culture conditions for cell production from juice of citrus waste peels. *Jap. Soc. Fd Sci. Technol.* **27**(5), 213 - 220.

Oso, B. A. (1978) The production of cellulase by *Tolaromyces emersonnii*. *Mycologia* **LXX** (3), 577 - 585.

Reese, E. T. & Mandels, M. (1963) Enzymatic hydrolysis of cellulose and its derivatives. In *Methods in carbohydrate biochemistry.* Vol. II (ed. Roy L. Whistler), pp. 139 - 141. New York: Academic Press.

Snyder, H. E. (1970) Microbial sources of protein. *Adv. Fd. Res.* **18**.

Solomons, G. L. (1983) Single cell protein. *CRC critical Rev. Biotechnol.* **1** (1), 21 - 58.

Trilli, A. (1977) Prediction of costs in continuous fermentations. *J. appl. Chem. Biotechnol.* **27**, 251 - 259.