Effects of Various Inorganic Nitrogen Sources on the Growth and Biomass Production by *Candida utilis* Isolated from Fermenting Cassava Tubers

[1]Nwokoro, O., [1]Ogbonna, J. C., [1]Okpala, G. N., [2]Ubani, C. S. and [1]Anya, F.
[1]Department of Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria
[2]Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria

Corresponding author: Nwokoro, O. Department of Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria. **Email:** ogb554@yahoo.co.uk **Phone:** 234-8034402414

Abstract

Candida utilis isolated from fermenting cassava tubers was cultivated in salt media containing sucrose as carbon source and different concentrations of ammonium sulphate, potassium nitrate, sodium nitrate and urea used individually as sole nitrogen sources. The yeast was grown in a 100 ml shaken culture and harvested after a 10-day fermentation period. Variations in media composition significantly (P≤0.05) affected yeast biomass production. Ammonium sulphate at 0.1% (w/v) concentration resulted in the best biomass production of the isolate. Concentrations above 0.1% (w/v) did not result in correspondingly greater biomass yield. Urea gave better yields at lower concentrations but concentrations above 0.07% (w/v) resulted in lower biomass yield. Sodium nitrate and potassium nitrate were inferior to ammonium sulphate and urea as nitrogen sources for yeast biomass production. A kinetic model based on the variations of these inorganic nitrogen supplementation, level of aeration and subsequent biomass production by the isolate was proposed.

Keywords: *Candida utilis,* Nitrogen supplementation, Biomass, Cassava fermentation, Yeast growth

Introduction

Natural fermentation of cassava is used to transform and preserve cassava products as well as increase the organoleptic properties of the final product. Cassava fermentation, also called retting involves the activities of yeasts belonging to the genus *Candida* (Brauman *et al*., 1996). Cassava contains mostly starch and sugar with low protein (Stupak *et al.,* 2006). The biochemical activities of yeasts on cassava results in yeast growth by assimilating the carbohydrate content of cassava with a resultant increase in its protein content due to the synthesis of yeast biomass in the form of single cell protein.

Candida utilis is an important food yeast (Lemmel *et al.,* 1979; Villas-Boas *et al.,* 2003; Rajoka *et al*., 2004). It is used for food fermentation because of its ability to grow rapidly using a variety of inorganic nitrogen sources (Zheng *et al.,* 2005; Bekatorou *et al.,* 2006; Rosma and Cheong, 2007). The significance of yeasts in food technology as well as in human nutrition as alternative source of protein makes the production of food grade yeasts extremely important.

Formulation of microbial culture media for the synthesis of microbial products is a very important aspect of microbial productivity. A nutrient which supports optimum microbial growth has obvious economic benefit in the formulation of culture media with the aim of producing the desired microbial product at a high efficiency. This paper describes the growth and biomass production of *Candida utilis* in various assimilation media under agitation.

Materials and Methods

Sample collection: Fresh cassava tubers (TMS 98/0505) were harvested after 8 months of planting from the National Root Crops Research Institute, Umudike, Nigeria. The cassava tubers were used immediately for the fermentation studies.

Isolation of yeast: Cubed cassava tubers (2 kg) were soaked in 1 L of tap water in glass containers for 96 h. Samples were withdrawn and serially diluted in 0.1% peptone water diluents. Serially diluted samples were plated onto potato dextrose agar (Oxoid, Ltd., UK) plates containing 50 mg chloramphenicol per litre to suppress bacterial contaminants (Ampe *et al.,* 1999). Plates were incubated at 28±2^{0}C for 48 h. *Candida utilis* isolated from the fermenting cassava tubers was identified based on the taxonomic scheme given by Lodder (1970) and de Hoog *et al.* (2000).

Growth media: The growth media contained the following: sucrose, 3g; Mg SO_4. $7H_2O$, 0.2g; NaCl, 0.1g; KH_2PO_4, 7.0g; K_2HPO_4 , 1.2g; $FeCl_3$, 0.05mg; $ZuSO_4$, 0.5mg; $CuSO_4$. $5H_2O$, 0.08 mg; distilled water to 1000 ml. The pH of the medium was adjusted to 5.3 by the addition of 1% sterile lactic acid. Four inorganic nitrogen compounds-ammonium sulphate, sodium nitrate, potassium nitrate and urea were individually employed as nitrogen sources. In a given series of fermentation flasks, each nitrogen source was added into the chemically defined medium at levels of 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2 and 0.3% (w/v). Yeast cells were cultured at 28 ± 2^{0}C with orbital agitation at 150 rpm in 250 ml Erlenmeyer flasks containing the growth media.

Determination of yeast cell growth: Yeast cell growth was followed by measuring the optical density of the isolates in broth at 600 nm using a Spertrumlab 23A spectrophotometer.

Determination of yeast biomass: For each cell dry weight determination, a 10 ml culture broth in a

tube was centrifuged (Gallenkamp Centrifuge) at 5000rpm for 15 minutes at room temperature (28± 2^0C) and washed first with 0.2 M phosphate buffered saline (pH 5.3) and again with sterile distilled water. The cell paste was dried at 105^0C to constant weight in an oven. After cooling, the tube was weighed and the cell dry weight was calibrated from a predetermined standard curve prepared for micrograms yeast suspension against absorbance. Reported results are the mean values of duplicate determinations. The effects of the inorganic nitrogen sources on yeast growth were compared by the analysis of variance (ANOVA) whereby P≤ 0.05 was considered statistically significant.

Results and Discussion

Candida utilis isolated from fermenting cassava tubers was grown in media containing various concentrations of ammonium sulphate, potassium nitrate, sodium nitrate and urea as sole nitrogen sources for biomass production. Data in Table 1 show the growth of the isolate in four commercial nitrogen sources used for the cultivation of yeasts.

Table 1: Changes in optical density during growth of *Candida utilis* in a carbon base medium containing different concentrations of inorganic nitrogen sources

Nitrogen sources	OD at 600nm		
	0 Day	5 Days	10 Days
Urea	0.050	0.058	0.064
KNO_3	0.050	0.051	0.052
$NaNO_3$	0.048	0.049	0.051
$(NH_4)_2 SO_4$	0.052	0.120	0.180

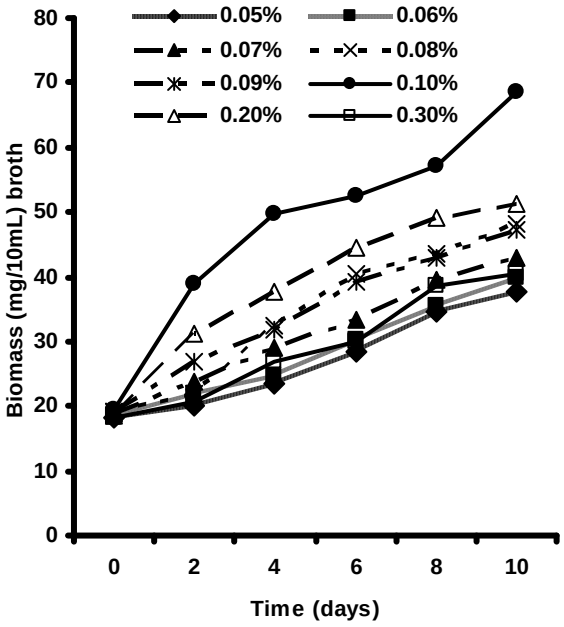

Fig. 1: Mean changes in biomass (mg/10mL broth) during the growth of *Candida utilis* in a carbon base medium containing different concentrations of ammonium sulphate

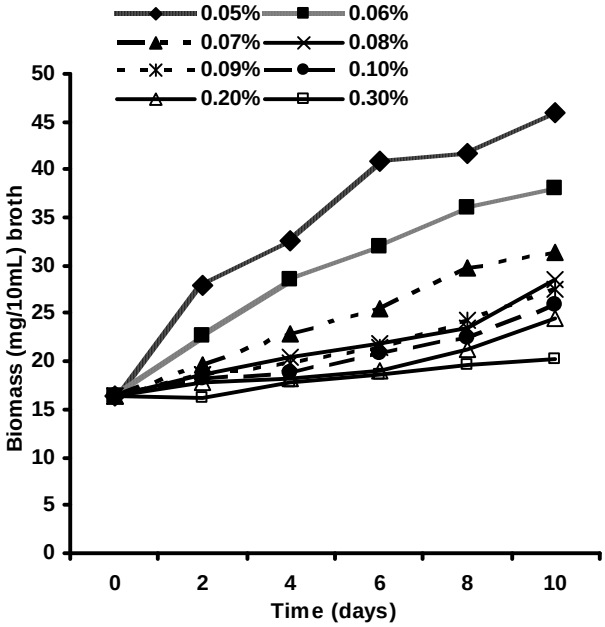

Fig. 2: Mean changes in biomass (mg/10mL) broth during the growth of Candida utilis in a carbon based medium containing different concentrations of urea as the sole nitrogen source

Ammonium sulphate is obviously the most favourable nitrogen source for culturing *Candida utilis* than urea which is better than potassium nitrate and sodium nitrate. The ability of some yeast to assimilate different inorganic nitrogenous compounds is an important diagnostic test for their classification (Lodder *et al.,* 1970; Harrigan and McCance, 1976; Bovill *et al.,* 2001). The most readily utilizable form of nitrogen by yeasts is ammonia (Harris, 1958; Sims and Folkes, 1964). All other nitrogenous sources are first reduced to ammonia before yeasts can utilize them (Pelczar and Reid, 1974). Results of test with ammonium sulphate (Fig. 1) showed that the increase in biomass of the yeast was highest at 0.1% (w/v) and lowest at 0.05% (w/v) level. Ammonium ions form a good nitrogen source for propagation of yeasts (Rose and Harrison, 1971). The metabolism of ammonium sulphate does not result in the production of toxic substances in the growth medium (Wickerham, 1946) but concentrations above 0.1% (w/v) did not result in correspondingly greater biomass production by the yeast (Fig. 1). This may be probably due to the inhibition of some enzyme systems at high concentrations of ammonium sulphate (Jiranek *et al.,* 1995). Our results are in agreement with those of (Chahal *et al.,* 1987) who reported maximum fungal biomass production from corn through fermentation with *Pleurotus sajor – cajo* in the presence of 0.14% (w/v) ammonium sulphate as the sole nitrogen source.

The metabolism of urea by the isolate resulted in a significantly lower (P<0.05) biomass production than with ammonium sulphate (Fig. 2).

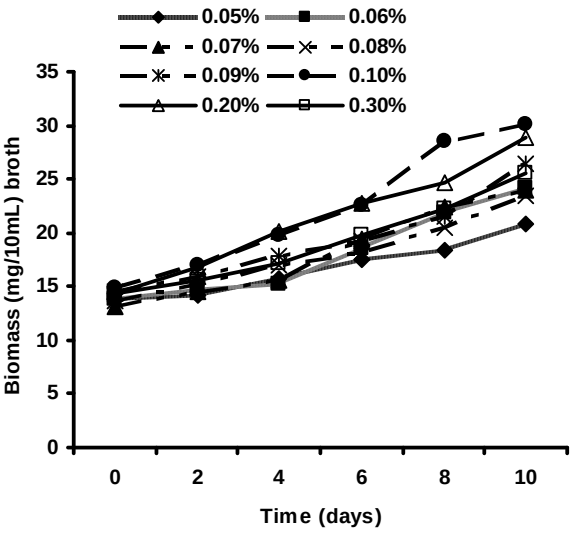

Fig. 3: Mean changes in biomass (mg/10mL) broth during the growth of *Candida utilis* in a carbon base medium containing different concentrations of potassium nitrate as sole nitrogen source

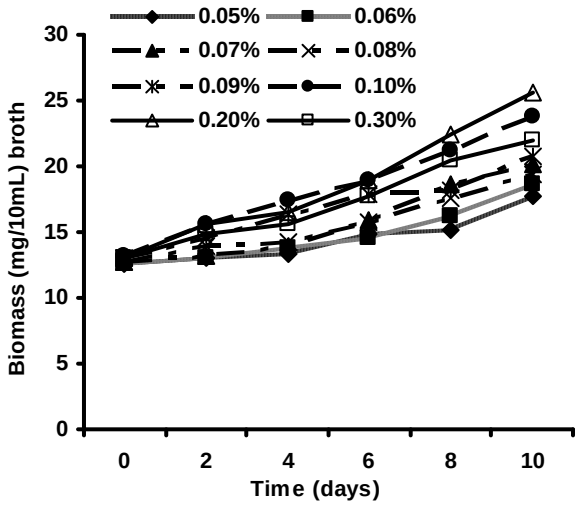

Fig. 4: Mean changes in biomass (mg/10mL) broth during the growth of *Candida utilis* in a carbon base medium containing different concentrations of sodium nitrate as the sole nitrogen source

Beneficial effects of urea on fungal growth were reported by Rainbault and Alazard (1980) who noted that urea hydrolysis librated ammonia. Wickerham (1946) reported that urea when used for yeast propagation resulted in the production of toxic substances which in high concentrations inhibited yeast growth, but such toxic substances were not produced during the assimilation of ammonium sulphate. This may be the reason for greater yeast biomass production associated with assimilation of ammonium sulphate than with urea. Practically, many yeasts can utilize urea in low concentrations as sole source of nitrogen (Waheed and Castric, 1997). Nevertheless, yeasts differ in their ability to hydrolyze high concentrations of urea. Our findings revealed that urea at concentrations of 0.05% (w/v) gave better biomass yield; concentrations above 0.07 % (w/v) did not result in good biomass productivity.

Comparatively, sodium and potassium nitrate were inferior to ammonium sulphate and urea as nitrogen sources for yeast biomass production. This finding is in agreement with the work of Anupama and Ravindra (2001) who also reported that ammonium sulphate gave higher biomass of *Aspergillus niger* when compared with supplementation with sodium nitrate. Incorporation of potassium nitrate into the growth medium at concentrations of 0.1% (w/v) gave a better biomass production than at a concentration of 0.05% (w/v). Also sodium nitrate at a concentration of 0.2% (w/v) resulted in a better biomass yield than at 0.05% (w/v). The inability of our isolate to use sodium nitrate and potassium nitrate for maximum biomass productivity may probably be due to the functioning of the enzyme nitrate reductase which converts nitrates to nitrites from which ammonia is produced (Garret and Amy, 1978; Cannons *et al.,* 1986). Further investigation is needed to the means of balancing the level of aeration and the extent of inorganic nitrogen supplementation to maximize the efficiency of inorganic nitrogen utilization by the yeast.

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