DISCUSSION

When yeast SCP production is to be achieved on the village level by fermenting cassava as proposed by Brook et al., (1969) and Nicol (1974), it becomes essential that the yeast itself performs hydrolysis. About 25 per cent (89) of approximately 400 currently recognized species of yeast are able to use starch as a source of carbon and energy. But, out of these 89 strains, the maximum conversion (hydrolysis) of starch was reported by 2 strains of Lipomyces konoenkoae as almost cent per cent, but with a specific growth rate of 0.12^{h-1} only (Spencer-Martins, et al., 1977).

In the present studies attempts were made to employ a strain of edible yeast for the fermentation of cassava meal and as very much wished a strain of Candida tropicalis was found to be capable of growing on soluble starch as well as cassava meal. Spencer-Martins et al. (1980) have tried 13 species of Candida for their ability to utilize starch and found a maximum yield coefficient was shown by a species Candida oregonensis. Recently protein enrichment of corn and cassava by Candida tropicalis was tried by Azoulay et al. (1980) and they have reported an yield of 0.55 g of cells per g of metabolized starch but with a rate of substrate conversion of 40 per cent only. By fermenting cassava by a strain of Candida tropicalis

they could increase the protein content of cassava from 5 to 20 per cent.

In the present study, a rapidly growing strain (M_3) on cassava meal with a high amylolytic activity could be isolated from a slow growing population of Candida tropicalis M_2. These two strains did not show any dissimilarity with respect to their morphology. However, they could be differentiated from one another on the basis of their pH optima, Ks values, maximum specific growth rate and duration of lag phase. It seems that M_3 strain is a spontaneous mutant. However, in the absence of genetic studies, it is difficult to categorically state that M_3 strain is a mutant.

The rapid growth of Candida tropicalis M_3 on cassava meal as well as soluble starch with a very short lag phase is suggestive of their ability to utilize starch by degrading it by releasing carbohydrases immediately. The cells may be having sufficient levels of enzymes for releasing into the medium as they have already been acclimatized to the substrate during the preparation of inoculum. The ability of these cells to utilize starch along with their high specific growth rate (μ max 0.76^{h-1})

and high rate of conversion of substrate (92-95 per cent) make them highly suitable for the commercial production of SCP from cassava. The yield coefficient (Y) of 0.46 g of pure cells per g of cassava carbohydrate supplied (but, the yield coefficient on the basis of g of biomass produced per g of carbohydrate metabolised was found to be 0.53, Table 6) compares favourably with the yield coefficient of 0.5 g cells per g of starch supplied found with several microbial systems (Spencer-Martins et al., 1977). It seems that the strain may be utilizing only the amylose part of the starch which amounts to 90-96 per cent of the total composition of cassava starch (Gopinathan Nair, 1981) and the β-limit dextrin liberated from the amylopectin may be remaining unutilized.

The permissive temperature for the growth of Candida tropicalis M_3 on cassava meal ranged from 34-38°C with an optimum at 36°C (Fig. 8). The optimum temperature of 36°C, with a permissive fluctuation of 2°C, for the growth of these cells also highly favours their use in the fermentation of cassava into SCP in tropical countries. The activation energy of growth (50 K Cals/mole. ° K, Fig. 10) of Candida tropicalis M_3 on cassava meal is comparable to that of other yeasts on various substrates (Saini, 1981).

The optimum pH 4 for growth (Fig. 11) being in the acidic range, it allows the fermentation in non-asceptic conditions (as observed in the present work) as the bacterial contaminations at this pH was found to be absent or if present, only in negligible levels.

The optimum rate of aeration for the maximum growth of Candida tropicalis M_3 was found to be 0.235 VVM and above (Fig. 13). However, some growth was observed even without aeration and this can be due to the inbuilt oxygen present in the substrate along with the small amount of O_2 present in the fermenter.

At sub-optimal concentrations of substrate, the specific growth rate of Candida tropicalis M_3 and M_2 was found directly proportional to the concentrations of the substrate upto a critical concentration (Tables 11 and 12) and thereafter the specific growth rate attained the maximum. These results are in line with any other microbial systems. The saturation constant (Ks) of 0.125 g/l (Fig. 15) favourably compares with many microbial systems grown on carbohydrates (Malek et al., 1969). The saturation constant of Candida tropicalis M_3 (0.125 g/l) is remarkably high as compared to that of

Candida tropicalis M_2 (0.008 g/l; Fig. 14). This may be due to the fact that rate of starch degradation is governed by the available activity of starch hydrolysing enzymes (amylases) elaborated by the cells for which the substrate acts as an inducer and the concentration of which may be a limiting factor for the growth rate of Candida tropicalis M_3 on cassava.

In batch fermentations, it was found that the autoclaved growth medium containing cassava meal contains other than the 1 per cent reducing sugar, only negligible quantities of glucose (less than 0.2 per cent) which might be liberated by starch hydrolysis during autoclaving. It could, therefore, be concluded that this strain Candida tropicalis M_3 grows directly on cassava meal (starch) and not on previously hydrolyzed starch. This clearly proves extracellular degradation of starch by carbohydrases as reported by Spencer-Martins (1977) and Lammel et al. (1980). However, these results differ from those of Azoulay et al. (1980). They could not observe any activity of extracellular amylases.

The stability of the strain Candida tropicalis M_3 was confirmed by conducting semi-continuous fermentation for 247 hours.

The results of semi-continuous culture (Fig. 16; Tables 8, 9 and 10) are found to be similar to that of a typical continuous culture of any microbial system. This proves that the efficiency of semi-continuous culture is as good as that of a continuous one. At low dilution rates (upto 0.4^{h-1}), as it approached the maximum dilution rate, the cell concentration decreased sharply as would be expected in a continuous culture (Fig. 16; Table 8). In Fig. 17, it can be seen that the theoretical values of cell concentration coincide with the observed values upto a dilution rate of 0.4^{h-1}. This suggests the correctness of determination of Ks value of Candida tropicalis M_3 grown on cassava meal. The deviation of theoretical values from observed ones beyond the dilution rate of 0.4^{h-1}, may be due to the variations in the yield coefficient of the strain which was assumed as constant.

The maximum biomass productivity of Candida tropicalis M_3 on cassava in semi-continuous culture was observed at a dilution rate of 0.57^{h-1} (Fig. 16). But at this dilution rate the percentage of conversion of substrate was only 77 (Table 8). For achieving high productivity (near maximum) along with high rate of substrate conversion (85.6 per cent), a dilution rate of 0.5^{h-1} should be

maintained (Table 8). For maximum conversion of cassava meal (92 per cent) into SCP, fermentation should be carried out at a dilution rate of 0.4^{h-1}, but at the cost of productivity.

The pattern and level of activities of extra and intra-cellular amylases (α and β) and amyloglucosidase observed under the influence of cassava meal and soluble starch (growth medium) clearly suggest that Candida tropicalis M_3 utilizes these substrates in the same way.

The observed high activities of extracellular amylases (Figs. 18 and 19) and amyloglucosidases (Fig. 23) clearly suggest the hydrolysis of the substrate (starch or cassava meal) by the yeast itself. Similar extracellular hydrolysis of substrate by yeasts has been reported by Spencer-Martins et al. (1977) and Lammel et al. (1980). However, Azoulay et al. (1980) could not observe any extracellular hydrolysis of the substrate (Starch and cassava) by a strain of Candida tropicalis. In the present studies, more than 90 per cent of total amylolytic activity has been found due to β-amylase (Figs. 18 and 19). β-amylase has been reported as the major amylase in several species of Bacillus (Honsley

et al., 1980; Shinke et al., 1980; Murao et al., 1979; Ueda, 1980; Takasaki et al., 1976). The high activity of β-amylase can be correlated with the high content of amylose in cassava starch as β-amylase can completely convert amylose to maltose. The observed high activity of extracellular amyloglucosidase also enables the hydrolysis of maltose, formed as a result of the activity of amylases, to glucose. The glucose thus formed can be used for the growth of the cells.

The activity of extracellular β-amylase increased during the exponential phase of growth of Candida tropicalis M_3 and declined thereafter (Figs. 18 and 19). This diminution of activity may be due to a feedback inhibition. The observed increase in the activity of extracellular amyloglucosidase even after the exponential phase (Fig. 23) of growth clearly suggests the high content of maltose in the broth which may be inhibiting the activity of extracellular β-amylase. A rapid increase in the activity of extracellular acid protease (Figs. 24 and 25) observed after the exponential phase of growth also may be responsible for the reduction in the activity of extracellular β-amylase (Rainbow and Rose, 1963).

The biomass of fermentation has been found to contain

high levels of nutritional factors such as 47 per cent of protein, 34 per cent of carbohydrate, 4 per cent of lipids. The pure cells separated from the final biomass showed a protein content of 58 per cent (Table 13). Cell protein has been found to be relatively balanced in its essential amino acid composition (Table 14). This protein has been found to be rich in lysine (4.25 g/16 g N_2) and methionine (0.66 g/16 g N_2). The processed fermentation biomass of cassava meal has got a light cream colour and smooth texture. It is worth mentioning that the product (SCP) doesn't have any unpleasant odour.

The absence of toxicological and acceptability tests of the product (SCP) restricts its recommendation for human consumption. However, taking into consideration the long history of use of Candida tropicalis grown on carbohydrate as food yeast, it can be taken for granted that the SCP obtained by fermentation of cassava meal by Candida tropicalis M_3 is suitable for human consumption.

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