SUMMARY AND CONCLUSION

The global demand for food protein is rapidly increasing with the rapid increase in world population. Taking into consideration the rate at which the world population increases and the demand for proteins, the protein deficiency is estimated (estimated from the past production rate of proteins) to touch a figure of 13 million tonnes in 1985 and 22 million tonnes by the year 2000 (Hoshiai, 1981; W.B. World Development Report, 1980; U.S.A. The Global Report to President, 1980; FAO - The Fourth World Food Survey, 1977). For increasing the production of various protein sources, scientific human wisdom should be brought into full play. Though the deficiency circumstances will vary with countries, both developed and developing countries are commonly concerned in the essential necessity of coping with the deficiency without this effort, there is no solution to feed problems. Although even coping with the deficiency is already too late in view of the current world situation with the prevailed shortage of proteins, the longer it takes to cope, the more critical will be the problems. In this sense, single cell proteins appear to be sole product of present human wisdom by which feed proteins can be produced on a scale of millions of tonnes with the help of natural

microorganisms requiring no farms. If the deficit of 13 million tonnes of feed proteins in 1985 is to be made up with single cell proteins, the amount is equivalent to 22 million tonnes of single cell proteins, and similarly the deficit of 22 million tonnes of feed proteins in 2000 is equivalent to 37 million tonnes of single cell proteins (Kazuo-Hoshiai, 1981). One of the first considerations of SCP production is the choice of substrate. At present two major substrates used for the SCP production are hydrocarbons (n-paraffins) and ethanol. However, with the fast depletion of petroleum crude and the utilization of ethanol as motor fuel, a time has come to look for other cheaper and available substrates. In this context, classical carbohydrates can be a promising and cheaper substrates for the production of SCP as compared to the traditional sources. The two major sources of carbohydrate, especially for the production of starch, are maize (Zea mays) and potato (Solanum tuberosum). A third potentially high source of carbohydrate (starch) is cassava (Manihot esculenta Crantz.). Cassava is grown in more than 80 countries of the humid tropics of the world. India assumes the 5th place among the cassava producing of the world with a production of 5.2 million

tonnes per year. In terms of yield of starch per hectare it is potentially unrivalled and is also less costly as compared to maize and potato. Around 400 million people in different countries are using cassava as a staple food stuff. Even though, the carbohydrate is very high in cassava, its protein content is extremely low and cases of malnutrition when the main constituent of diet is cassava are well known.

Among the different microorganisms, yeast has been always preferred because they have their own advantages over bacteria for the following reasons in SCP production: (1) Ease of acceptability, (2) stability of cultures, (3) ability to grow at lower pH, thus reducing the chances for contamination, (4) relative ease of biomass recovery due to bigger dimensions, (5) low nucleic acid content.

The most commonly used yeast in single cell protein work belongs to the genus Candida formerly known as Torula.

Many workers have proposed yeast SCP production on the village level by fermenting cassava or other starchy

foods as means for improving the diet in underdeveloped countries. But a review of literature on the SCP production from cassava clearly shows that most of the works were aimed at the protein enrichment of cassava rather than using cassava as a substrate for SCP production except in Symba process. The highest protein content of biomass recorded so far using a single strain of yeast, is 20 per cent. The Symba process is a symbiotic fermentation using one species of yeast for the hydrolysis of starch and another species for the conversion of the hydrolyzed products into SCP. Since the conversion of cassava into SCP is not fully explored present studies were undertaken with a view to :-

(1) finding out a non-fastidious strain of yeast preferably belonging to food yeast which can utilize cassava efficiently without pre-hydrolysis,

(2) standardising conditions for the maximum production of SCP (Batch and semi-continuous) along with characterisation of the strain,

(3) working out the mechanism of utilization of the starch by the strain,

(4) determining the major components of biomass such as protein, carbohydrates, lipids and essential amino acids.

Among different species of yeast belonging to 3 genera Candida, Torulopsis and Saccharomyces screened for their growth on starch, only Candida tropicalis M_2 found growing. A fast growing Candida tropicalis M_3 was isolated from a population of slow growing Candida tropicalis M_2 by successive subculturing in a synthetic medium containing cassava meal or soluble starch as the only source for carbon and energy. Different bench scale batch fermentation studies were carried out using cassava meal as the substrate to optimise the conditions for the growth of Candida tropicalis M_3. It was found that a temperature of 36°C, pH 4, aeration of 0.235 VVM and above at a given agitation of 1500 r.p.m., highly favours the growth of Candida tropicalis M_3 on cassava meal. The specific growth rate of Candida tropicalis M_3 was not affected by the different supra-optimal concentrations viz. 1, 1.5 and 2 per cent of substrates tried.

Under optimum conditions of growth, in batch cultures

(bench scale) Candida tropicalis M_3 showed a high specific growth rate of 0.73^{h-1} with a very short lag phase. Under these conditions of growth, around 95 per cent of the substrate was found getting converted into SCP. The yield coefficient under the said conditions was found to be 0.46 g of pure cells per g of cassava carbohydrate supplied (0.53 g of biomass per g of metabolized substrate).

The activation energy for growth of Candida tropicalis M_3 on cassava meal was found to be 50 K Cals/ mole, °K. Candida tropicalis M_3 showed a maximum specific growth rate (μ max) of 0.76^{h-1} and saturation constant (Ks) Of 0.125 g/l whereas Candida tropicalis M_2 showed a μ max of 0.45^{h-1} and Ks of 0.008 g/l.

From semi-continuous fermentation studies of cassava meal by Candida tropicalis M_3 employing different dilution rates (upto 0.4^{h-1}) the cell concentration, percentage of substrate conversion and yield approach to that of batch fermentations. After a dilution rate 0.57^{h-1} and above, cell concentration, percentage of substrate conversion and yield were found to be sharply declining and at a dilution rate of 0.75^{h-1} complete washing out of cells was observed.

The maximum biomass productivity was observed at a dilution rate of 0.57^{h-1} ($\bar{DM}$). The stability of the strain Candida tropicalis M_3 was checked by semi-continuous fermentation for 247 hours.

Semi-continuous fermentation of soluble starch by Candida tropicalis M_3 was also carried out by employing 2 dilution rates viz. 0.1 and 0.6^{h-1}. Results of these studies showed the same trend as observed in the semi-continuous fermentation of cassava meal by Candida tropicalis M_3.

It has been observed that Candida tropicalis M_3 itself hydrolysis the substrate and the hydrolyzed product is used for its growth. The hydrolysis of the substrate has been found being carried out with the help of extracellular carbohydrases viz. amylases and amylo-glucosidase. More than 90 per cent of the total amylolytic activity has been found due to β -amylase. The activity of extracellular carbohydrases was found increasing almost parallel to the exponential phase of growth. The activity of intracellular carbohydrases was very low as compared to that of extracellular carbohydrases.

A very low activity of extra and intra-cellular acid protease was observed.

The SCP obtained by fermentation of cassava meal by Candida tropicalis M_3 was found to contain 47 per cent protein, 35 per cent carbohydrate and 4 per cent lipids. The essential amino acid profile of this SCP was found to be relatively balanced. The protein has been found to be rich in lysine (4.25 per cent). The pure cells separated from the final biomass contained 58 per cent of protein.

From the present studies, it can be concluded that :-

(1) an edible yeast, Candida tropicalis M_3 was found capable of efficiently converting cassava meal as well as soluble starch into SCP through a single step fermentation,

(2) a temperature of 36°C, pH 4, aeration of 0.235 VVM and above at a given agitation of 1500 r.p.m., have been found to be the optimum conditions for fermentation (batch and semi-continuous). In semi-continuous fermentations, the maximum biomass productivity with a substrate conversion rate of 77 per cent has been observed at a dilution rate

of 0.57^{h-1}. For a high rate of substrate conversion (85 per cent) and biomass productivity, a dilution rate of 0.5^{h-1} has to be maintained,

(3) very high activities of extracellular amylases and amyloglucosidase have been observed in the active period of growth. This clearly suggests that yeast itself hydrolysis the substrate.

(4) The biomass of fermentation (SCP) of cassava meal by Candida tropicalis M_3 has been found containing 47 per cent of protein with a relatively balanced composition of essential amino acids. In the absence of toxicological and acceptability tests, taking into consideration the history of Candida tropicalis grown on carbohydrates as an edible yeast, it can be said that the SCP is suitable for human consumption.

(5) The optimum conditions of growth (temperature 36°C, pH 4), high rate of substrate conversion and yield through single step fermentation and high specific growth rate highly favour the commercial production of SCP by the fermentation of cassava meal by Candida tropicalis M_3 under non-asceptic conditions in tropical regions.

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