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YEAST AS A SOURCE OF PROTEINS

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INTRODUCTION

The teaming human population poses an ever-increasing demand for new sources of protein. In fermented foods and beverages, the consumption of microbes is essential. In the 1960s, the recognition of a world-wide shortage of protein led to renewal of attempts to solve this problem by the large-scale production of biomass as a source of food. The main usage of microbial biomass is at present in animal feeds. However, a significant portion is being used in foods meant for human consumption.

The conscious growth of microbes for human consumption started in Germany about 1910 with brewers' yeast which was separated from the beer, washed (and sometimes debittered), and dried. During and following World War II, the yeast *Candida utilis* was grown on wood sugar containing sulphite liquors, a by-product of the paper industry.

The term single cell protein (SCP) has been widely applied to microbial cell material intended for use as food or feed. According to Wilson (1968) the term, single cell protein, has a certain psychological value since any unpleasant connotations with terms such as bacteria, fungi or microbial protein are thereby avoided. The term was coined at the Massachusetts Institute of Technology around 1966 as a substitute for names that implied "derivation from microorganisms or, worse still, from waste materials or petroleum products".

The importance of microorganisms as a potential source of food stems from the finding that microbial cell matter is rich in most B-group vitamins and in proteins that contain essential amino acids. They, therefore, constitute enrichments for deficient diets (Bhattacharjee, 1970). Proteins of microbial origin compare favourably with conventional sources such as egg, meat and fish in terms of overall pattern of nutrient (Miller, 1968).

According to Kihlberg (1972) microorganisms have the following general advantages over plants and animals as protein producers:

(i). Microorganisms have very short generation time, and can therefore provide a rapid mass increase. Under the most favourable conditions, bacteria and yeast have generation times of about 0.5 - 2.0 and 1.0 - 3.0 hours respectively, while algae and filamentous fungi are able to double their masses in 2 - 6 and 4 - 12 hours respectively. However, generation times for plants, chicks and sheep are at least 3 weeks, 5 - 8 weeks and 2 - 8 months respectively.

(ii) The production of microbial protein can be carried out in continuous culture, independent of climatic changes and with only a small land area and water requirement. Thus, the waste disposal problems are small compared with those encountered in most other processes for food production.

(iii) The raw materials for single cell production, e.g. cellulose, petroleum or natural gas, or coal are readily available and abundant in nature.

(iv) On a dry weight basis the protein content of microorganisms is higher than that for most common foodstuffs. Most microorganisms contain between 7 and 12% nitrogen, even after correction for non-protein nitrogen from purines, pyrimidines etc.
(v) Microorganisms can be easily modified genetically. Among the various microbes in use for single cell protein, yeasts, filamentous fungi, bacteria, and algae have been considered. Yeasts are the most important organisms for use as food. They are noted for their safety and the ability to separate them cheaply from the growth medium (by centrifugation).

2. Yeasts as a source of single cell proteins

Many different species can be used as a source of proteins and they differ in physical properties, cultural requirements and flavour (Cook, 1958). The last point is important because the initial lack of enthusiasm shown for yeast has been attributed to its intrinsic flavour which has been attributed to the anti-foaming agent used in making some batches. Some batches have caused digestive disturbances and animals have sometimes not thrived on such yeast. The trouble may be caused by microbial contamination, intact cell walls, the polyphosphates in fresh yeast and in other ways, different methods of handling may be needed to remedy each type of problem (Pirie, 1963).

Yeast is also one of the richest sources of the B-vitamins, and its role in animal nutrition as animal feed supplement (0.5 - 1.5%) has generally been to provide these vitamins (Bressani, 1968). For instance, *Candida* sp. serves as a good source of riboflavin and pantothenic acid (Bhattarcharjee, 1970). Some species of yeasts presently used in foods include: *Saccharomyces cerevisiae, Candida utilis, Kluyveromyces fragilis* and *Pichia membranaefaciens*.

S. cerevisiae, a top-fermenting brewer's yeast and also known as baker's yeast, is used in the fermentation of beer and also in bakeries. The yeast is rich in amino acids and vitamins. In addition to its primary usefulness in the brewing of beer, it is also important in human nutrition as a source of protein and vitamins in animal feed. Brewer's yeast derives from strains formerly known as *Saccharomyces carlsbergensis*, later *S. uvarum*, but now grouped under *S. cerevisiae*, the strains used in brewing larger beer. The bitterness of brewer's yeast to taste arises from the hops contained in the wort, and it must, therefore, be debittered.

The role of yeast in the baking industry is to leaven bread and related products. Two types of baker's yeast are on the market: compressed, with a moisture content of 70%, and activated dry yeast, containing 7.5% moisture. In the dry form, yeast is inactive and must be hydrated in warm water before the cell can grow. Dry yeast is more stable than compressed, and is consequently very useful in the tropics where bread making with compressed yeast is difficult to control.

3. General characteristics of yeast proteins.

The composition of microbial cells has been shown to be greatly affected by changes in the medium and culture conditions. Tatcher (1954) has shown that when nitrogen limits the growth, the cells may accumulate large amounts of lipids. However, Ierusalimsky (1962) also showed that the relative amounts of synthesized protein and fat depend upon the C:N ratio of the medium.

A table published by Kihlberg (1972) showed the gross chemical composition of yeasts in comparison with other classes of microbes It can be seen from this table that there are many deviations from the mean values. This is in agreement with an earlier report by Evans (1968) that some hydrocarbon derived yeasts contain more nitrogen (10 - 11%) than what is generally encountered.

The average elemental composition of yeast biomass as determined by Peppler (1970) is as follows: carbon, 45 - 47%; oxygen, 31 - 32%; nitrogen, 7.5 - 9.0%; hydrogen, 6.0 - 6.5%; ash, 8.0%. The biomass of unicellular yeast-like organisms contains usually 40 - 60% protein, depending on the strain and conditions of growth. In comparison with other protein

sources,Cartwright (1958) had earlier shown that yeast has a much greater potential for protein production than conventional food sources. Apart from bacteria which usually contains up to 70% protein, beef (cattle), poultry, cattle milk and swine were shown to contain between 4 and 20% protein (Tannenbaum and Wang, 1975).

Kihlberg (1972) analysed the chemical composition of yeasts and showed that true protein content is generally only 40 to 48% because about 15% of the nitrogen is present in the form of non-protein materials such as nucleic acids (Table 1). High nucleic acid content is associated with rapidly growing cells. Compared with conventional food, the yeast thus contains a high amount of nucleic acids even when expressed on a protein basis, namely, between 8 and 25g nucleic acids per 100g protein (Kihlberg, 1972). The generally accepted safe level of nucleic acid intake in the human diet has been shown to be 2g per day (Cooney *et al*., 1980), equivalent generally to 20g of yeast. The ingestion of high nucleic acid compounds is associated with high levels of uric acid in blood because of the absence of uricase. High concentrations of uric acid can cause gout; stones may also form in the kidneys and bladder.

Table 1: Gross chemical composition (%) of yeasts in comparison with other classes of microbes.

Constituents	Percentage composition			
	Filamentous algae	Algae	Yeasts	Bacteria
Nitrogen	5.8	7.5 - 10.0	7.5 - 9.0	11.5 - 13.3
Crude protein (N x 6.25)	31.50	47 – 63	47 - 56	72 - 83
Nucleic acids	9.2	3.8	6 - 12	8 - 16
Ash	9 - 14	8 – 10	5 - 9.5	3 - 7
Lipids	2 - 8	7 – 20	2 - 6	1.5 - 3

(Ref: Khilberg, 1972)

The nitrogen balance of yeast biomass depends on the production medium. Fodder yeast is usually propagated on various industrial or agricultural wastes which cannot be utilized for other purposes. As an adjuvant of fodder mixtures, yeasts have an additional advantage of containing antioxidants which prevent lipid components of the fodder from turning rancid. The importance of yeast as a potential source of food arose from the finding that yeast cells as a potential source of food arose from the finding that yeast cells also have a high content of vitamins, mostly of the B complex, and in protein that contains essential amino acids (Kokova-Kratochvilova, 1990). The quality of biomass of different yeast species compared well with conventional sources such as egg, milk, meat and fish in terms of overall pattern of nutrients (Miller, 1968).

Carter and Phillips (1944) had earlier shown that all of the essential amino acids except methionine and cystine (sulphur-containing amino acids) are present in adequate quantities in yeast protein.

Table 2 shows that the essential amino acids present in *S. cerevisiae* and *C. lipolytica* compared well with those of wheat and egg. Also, there is evidence of a well balanced amino acid pattern with low content of S-containing amino acids as the most obvious deficit.

Table 2: Essential amino acid content of wheat, egg and two other yeast species.

Amino acids	*S. cerevisiae*	*C. lipolytica*	Whole wheat	Whole egg
Lysine	7.7	7.8	2.8	6.5
Threonine	4.8	5.4	2.9	5.1
Methionine	1.7	1.6	1.5	3.2
Cysteine	-	0.9	2.5	2.4
Tryptophan	1.0	1.3	1.1	1.6
Isoleucine	4.6	5.3	3.3	6.7
Leucine	7.0	7.8	6.7	8.9
Valine	5.3	5.8	4.4	7.3
Phenylalanine	4.1	4.8	4.5	5.8

Values are expressed in g/16g nitrogen.
(Ref: Khilberg, 1972)

Kockova-Kratochvilova (1990) compared the quality of biomass of different yeast species by cultivating representatives of individual genera on malt agar. According to the relative proportion of individual amino acids in their biomass, various types of yeast are divided into groups based on mutual correlation. The main difference in this respect was found between the *Ascomycetes* and *Basidiomycetes*, the latter having a lower proline and methionine content. A positive correlation was also found between the content of proteins and that of nucleic acids, especially RNA. Table 3 shows the concentrations of nitrogen compounds in some yeast species cultivated on celophane foils by Kockova-Kratochvilova (1990).

Nutritional studies by Bhattacharjee (1970) on yeast have revealed that crude protein accounts for 45 - 55% of dry weight. He also performed feeding experiments with rats and observed that yeasts were readily digested and absorbed, and that they provided up to 94% of the calorific value of the diets. Also, feeding experiments with dogs and man showed utilization of 80 and 90% respectively. To be of any nutritional values, yeasts must be killed and dried because live yeasts are known to deplete intestinal B vitamins of mammals, thereby causing avitaminosis (Bhattacharjee, 1970).

Cereal grains are known to contain low amounts of protein which are also deficient in lysine. Enrichment of corn-meal with dried food yeast has been attributed to lysine and tryptophan of yeast protein (Sure, 1948). Also, human subjects have been used to demonstrate the increase in biological value of white bread supplemented with DL-lysine or dried yeast (Bressani, 1968).

Before using biomass as a food adjuvant for human nutrition it has to be processed, usually by disintegration and cell walls removed by centrifugation. At higher doses and on prolonged intake the cell walls could cause liver disorders (Edozien *et al*., 1970). Removal of cell walls can be efected by mechanical disintegration of the cells or by cell autolysis at elevated temperature which includes the action of proteolytic and hydrolytic enzymes of the cells themselves. These enzymes have different effects on *S. cerevisiae* and *C. utilis* because of the ability to produce extracellular RNAse. For example, Kockova-Kratochvilova (1982) observed that 70% of *S. cerevisiae* strains cannot produce extracellular RNAse whereas 86% of *C. utilis* strains produced extracellular RNAse. For this reason, autolysis can be used in *C. utilis* also for lowering RNA content; while in *S. cerevisiae* this would require a considerable extension of the time of autolysis which would cause a substantial decrease in protein content because of the action of proteolytic enzymes. In order to circumvent this effect, autolysis in baker's yeast is usually stopped by cell disintegration, followed by incubation in the presence of sodium chloride (Gierhaz and Potter, 1979).

Table 3. Amino acid composition of yeast proteins (mol %)

Amino acid	*Saccharomyces cerevisiae* CCY-21-4-13	*Candida utilis* CCY-29-38-18	*Pichia farinosa* CCY-39-18-3	*Torulopsis kruissi* CCY-26-19-7	*Hansenula anomala* CCY-38-1-1	*Schizosaccharomyces pombe* CCY-44-1-3
Lysine	7.15	6.33	5.74	7.54	7.93	6.16
Histidine	2.36	1.57	1.18	2.62	2.48	2.53
Arginine	6.16	3.24	2.19	3.86	4.18	7.59
Aspartate	9.32	10.58	11.49	9.03	9.47	8.35
Threonine	4.45	7.63	5.36	6.90	5.51	5.42
Serine	5.41	9.30	10.03	7.61	7.32	7.28
Glutamate	17.60	16.35	13.10	12.88	12.09	11.57
Proline	5.97	3.45	8.48	7.15	6.65	5.24
Glycine	8.54	9.08	8.67	7.70	7.93	8.65
Alanine	7.46	10.01	9.36	9.55	9.86	8.90
Valine	6.09	3.63	4.61	6.21	5.91	5.56
Methionine	1.18	0.85	0.71	0.81	1.08	1.50
Isoleucine	5.87	4.07	4.15	4.56	5.38	6.33
Leucine	6.54	4.07	7.53	7.16	7.85	7.83
Tyrosine	2.63	2.87	3.10	2.86	2.78	3.28
Phenylalanine	3.25	3.20	3.68	3.53	3.56	3.79

Ref: Kockova-Kratochvilova (1990).

Table 4. Solubility of n-alkanes in water at 25°C

n-Alkanes	Saturated solutions (mol/l)
Hexane	1.1×10^{-6}
Octane	5.8×10^{-6}
Decane	3.1×10^{-7}
Dodecane	1.7×10^{-8}
Tetradecane	9.8×10^{-10}

Ref: Einsele and Fletcher, 1971

23

4. Raw materials for producing yeast single cell protein

The raw materials which are used for yeast SCP production can be divided into two categories, viz. yeast cells and substrates for cultivation. Industrial cultivation of yeast biomass can be divided into traditional and non-traditional substrates.

The most popular microorganisms for food production are the yeasts. They have been used as fodder for certain animals such as horses and cows, and as feed for poultry. Cooney *et al*. (1980) have suggested that when fed to cows, yeasts will increase the production of high quality milk. Since baker's yeast is also added to flour at a concentration of about 1% (w/w), it represents an important source of microbial biomass in human diets (Maiorella *et al*., 1981).

Yeast cells are much larger than bacteria and are much easier to harvest. The yeasts are also more familiar to human experience. They have been used in foods as vitamin additives and flavouring agents for a long time; they have reasonable protein contents (Table 1) and are easier to recover from fermentation broths than bacteria. Some yeasts such as *S. cerevisiae* (brewer's and baker's yeast), *Kluyveromyces fragilis*, and *Candida utilis* have long been used in food industries. Other yeast species being used for SCP production belong to the genera *Rhodotorula, Torulopsis, Pichia* and *Schizosaccharomyces* (Kokova-Kratochvillova, 1990).

C. utilis (Torulopsis utilis) is a *Torula* yeast which has had some success in human feeding but is produced mainly for inclusion in animal feeds. One limitation of yeast for human feeding is that the S-containing amino acids are present in low amounts. *C. utilis* grows well on sulphite waste liquor which provides a disposal problem for paper mills (Peterson, 1978).

C utilis exhibits polyauxia, i.e. gradual utilization of several substrates. Ghose *et al*. (1977) showed that the utilization of one substrate may be inhibited by repression of enzyme synthesis and also by inhibition of transport activity. A relatively large variability exists among industrial strains of *C. utilis*. Evaluation of strains of the yeast by Poliachova (1978) showed that different groups were observed which differ in cell size, ability to utilize molasses and potato mashes, and other compounds such as citric acid, succinic acid, DL-lactic acid and glycerol.

Brewer's yeast is a heterogeneous group of yeasts which have been classified into two taxonomic groups: *Saccharomyces uvarum*, (formerly *S. carlsbergensis*), is capable of producing the extracellular enzyme α-galactosidase and is therefore able to ferment melibiose; and *S. cerevisiae* which includes strains negative in this regard.

Traditionally, *S. uvarum* was used in the production of larger beer by bottom fermentation while *S. cerevisiae* was used in the production of ale, usually by top fermentation at higher temperatures. However, the difference between these two groups has been considered insufficient to justify their separation into two species. Both of them have therefore been consolidated within the species *S. cerevisiae* (Barnett *et al*., 1983; Kreger-van-Rij, 1984).

Brewing yeast (*S. cerevisiae*) are polyploid or aneuploid (Molzahn, 1977; Aigle *et al*., 1984). Unlike laboratory haploid strains of yeast which have been subjected to constant selection or regular sexual reproduction and high spore viability, brewing yeast lack a mating type. They are reticent to mate and exhibit low spore viability (Johnston, 1965; Fowell, 1969; Anderson and Martin, 1975). These properties confer a measure of phenotypic stability which makes them less susceptible to the destabilizing forces of mutation and recombination.

Saccharide substrates are utilized for producing yeast SCP. Various starch-containing waste products, molasses, beet juice and sulphite liquors belong to the traditional waste substrates used for the culturing of fodder yeast. The main yeast grown on these substrates is *Candida utilis*. According to Kihlberg (1972), starch from tapioca, cassava, potato waste etc., can be fermented by a single-step continuous symbiotic process in which *Endomyces filburger* hydrolyses the starch to sugar which are then utilized for

biomass production by *C. utilis*. The yeast is also grown on various waste waters, e.g. from the production of citric acid. The water from citrate production contains on average peak concentrations of 2.5 to 7.0% utilizable saccharide. For cultivation purposes the water is supplemented with nitrogen, phosphorus, potassium, magnesium and sulphur. Leopold and Spanelova (1974) have found that *C. utilis* does not require vitamins if the concentration of saccharides does not exceed 1.5%. At higher saccharide concentrations the yeast requires mainly biotin and inositol which can be added in yeast extract, potato liquor, beet juice, etc.

Kotti (1984) has suggested that molasses, which are waste products of the sugar industry, are good substrates for the cultivation of baker's yeasr. There is need, however, to provide a good source of nitrogen in the medium. Urea is utilized as the nitrogen source. Vitamins such as biotin and inositol are also supplied by yeast extract. Phosphorus is supplied in the form of sodium dihydrogen phosphate. Urea is now being employed because it has been found superior to ammonium salts in terms of yield on dry weight basis (Rose, 1972). Aeration is provided to prevent alcohol production.

Other substrates are represented by hydrolysates of solid wastes such as animal excrements, fibrous wood products, straw, corn cones, tree bark and twigs, saw dust, wood chips, etc. Fibrous products obtained by delignification of wood, which are used in the paper and chemical industries contain, in addition to cellulose, hemicellulose, residues of lignin and other substances, and they have been correctly called pulp. According to its properties, pulp is divided in practice to α-, β-, and γ-celluloses. α-Cellulose is a fraction of pulp insoluble in 18% NaOH and it represents pure cellulose.

β-cellulose is a degradation product of cellulose, originally absent from wood. It is dissolved in alkali and can be precipitated back by acidification. Cellulose represents the hemicellulose fraction of pulp; it is soluble in alkali but cannot be isolated from the solution by acidification. The hydrolysis of solid wastes is carried out chemically or with the aid of enzymic degradation. For instance, cellulose is conveniently degraded by *Trichoderma viride* or *T. reesei*. The main disadvantage of chemical hydrolysis is that their content of a number of inhibitors of yeast metabolism and reproduction which have to be removed before cultivation, thus raising the cost. Yeast propagation is also hampered by caramelization which occurs at higher temperatures.

The ability of microorganisms to decompose hydrocarbons has long been known, but the utilization of this ability for the production of fodder biomass is relatively recent. The hydrocarbons are non-traditional substrates.

The tremendous value of hydrocarbons as substrates lies in their abundance and relatively low cost. Yeasts belonging to the genera *Debaryomyces, Hansenula, Torulopsis* and *Candida* are capable of fermenting hydrocarbons (Yamada *et al*., 1968). *C. tropicalis* has been successfully cultivated in a hydrocarbon medium at a very high temperature. Hydrocarbons such as n-paraffins are excellent substrates for yeasts. Growth is rapid in well-aerated cultures, and the yield of protein and essential amino acids is excellent. Also, yeast species belonging to some genera, e.g. *Hansenula* and *Candida* utilize solid paraffin as a carbon source (Peterson, 1978). However, solid paraffin or paraffin wax has only rarely been used for fermentation, while liquid and gaseous hydrocarbons are being used for this purpose on a large scale. In 1967, Wagner patented a method of fermentation of solid hydrocarbons. Earlier, Miller and Johnson (1966) described fermentation by *Candida intermedia* and *C. lipolytica* strains utilizing C_{22} to C_{28} hydrocarbons as well as paraffin wax which yielded a rich biomass. Paraffin wax is an oil component obtained as a product in the production of fractions of medical paraffin oil and motor oil from crude oil. It contains 91% normal paraffins ranging between C_{25} to C_{37} (Yamada and Yogo, 1970). Yeasts can thus be used for dewaxing oil fractions.

The utilizability of hydrocarbons also depends on their solubility in water. The solubility of short-chain hydrocarbons was found to be inversely proportional to the chain length, but the proportionality

coefficient decreases with increasing chain length as shown by Einsele and Fischer (1971) (Table V).

Yeast cells produce biomass on alkanes only when the aliphatic chain contains at least eight carbon atoms. The most rapid utilization is found with hydrocarbons with C_{12} to C_{14} while the slowest utilization is with hydrocarbons which have more than 18 carbon atoms. The utilization of alkanes with short side methyl groups is very slow.

Alkyl-aromatic hydrocarbons with side chains shorter than C_5 are toxic for yeast cells. Production of biomass from branched hydrocarbons depends not only on chain lengths but also on the position of branching. For instance, isoalkanes branched at one end yield less than 50% biomass as compared with conventional alkanes. 8-Methylpentadecane yields only 20% biomass as compared with hexadecane as a substrate of equal molecular weight.

The two most acceptable pathways for the metabolic conversion of hydrocarbons are those earlier proposed by McKenna and Kallio (1965). generally, alkanes are converted to primary alcohols and aldehydes which then form correspondingly carboxylic acids. The carboxylic acid can subsequently be decomposed by β-oxidation or can again be further oxidised to dicarboxylic acid which is then decarboxylated. The first oxidation takes place in position C_1 or C_2 and involves an equilibrium between radicals of these two positions.

From studies devoted to the microstructure of yeast grown on n-alkanes as the only sources of carbon, Kawamoto *et al*. (1978) concluded that the microsomes are likely to take part in the biosynthesis of C4 compounds from acetyl CoA during assimilation of n-alkanes by the yeasts. Carboxylic acids are synthesized from n-alkanes in the cytoplasm. They are incorporated into the microsomes where they are activated to corresponding CoA esters. An oxidase or a certain flavoprotein catalyzes acyl CoA to molecular oxygen and hydrogen peroxide. Catalase decomposes hydrogen peroxide to water and molecular oxygen. Acetyl CoA synthesized during β-oxidation goes through the glyoxylate cycle. Malate and succinate are transported into the mitochondria where they are further metabolized. The production of C_4 compounds from acetyl CoA in the glyoxylate cycle requires a cooperation of microbodies with mitochondria because the latter have insufficient enzymes to perform the whole process.

Baker's yeast (*S. cerevisiae*) has long been known to exhibit diautic growth that reflects the utilization of added carbon sources, usually saccharide, followed after its exhaustion by utilization of the produced alcohol. The cells oxidise alcohol to acetate and then to carbon dioxide.

Prompted by the prospect of yeast biomass production from synthetic ethanol, the oxidation of alcohols including glycols by yeasts has been intensively studied. Considerable attention was paid to alcohol dehydrogenase (EC 1.1.1.1), its isolation, purification and physical properties. Alcohol dehydrogenase has been studied in detail in *S. cerevisiae* which grows well on ethanol as the sole carbon source. The enzyme was found to exist in several forms. Heick (1972) described three NAD-dependent alcohol dehydrogenases in baker's yeast. Also, a potent producer of biomass named *Candida ethanolica* was discovered by Rybcrora *et al*. (1980)

Methylotropic yeasts have the ability to utilize methanol as the sole source of carbon and methylamine as the sole nitrogen source. Methanol, a cheap carbon source, is a major by-product of the oil refining process. The ability of yeasts to utilize methanol in concentrations of 0.4 to 4% has been used to produce single cell proteins. The industrial potential of methylotropic yeasts initially arose out of their ability to utilize methanol as a carbon source. The production of single cell protein from methanol has successfully been commercialized as a feedstock additive, "Provesta", by Philips Petroleum Company.

Yeasts that are capable of utilizing methanol for biomass production belong mainly to the genus *Hansenula* (Hazeu *et al*., 1972). Levine (1973) analyzed the amino acid composition of the proteins in the yeasts and found them to be extremely suitable for the nutrition of higher

organisms. Taxonomically the yeasts are closely related (Ogata *et al*., 1969) and this is reflected in the consistent uniformity of the metabolic pathways in various yeasts. A key enzyme of the pathway, alcohol oxidase, can represent 30% of the total cell protein under optimal conditions. This enzyme is sequestered in peroxisomes and is subjected to strict regulation, being repressed by glucose and ethanol, and induced by methanol.

In methylotropic yeasts, methanol is oxidized to CO_2 via a linear pathway as shown by Douma *et al*. (1985) (Fig. 1). The oxidation is carried out by alcohol oxidase using molecular oxygen as electron acceptor within the peroxisome to produce formaldehyde and hydrogen peroxide. Hydrogen peroxide is reduced to water and molecular oxygen by catalase. Formaldehyde may diffuse into the cytosol where it spontaneously reacts with reduced glutathione. Formaldehyde dehydrogenase and formate dehydrogenase respectively oxidize formate-reduced glutathione complex first to formate and then to CO_2. An alternative pathway is the reaction of formaldehyde with xylulose-5-phosphate by the action of dihydroxyacetone synthase within the peroxisome to produce glyceraldehyde-3-phosphate and dihydroxyacetone. Dihydroxyacetone can be phosphorylated by the action of dihydroxyacetone kinase in the cytosol to produce dihydroxyacetone phosphate.

To generate biomass, entry of formaldehyde (one carbon) into the assimilatory pathway via the xylulose-5-phosphate cycle (five carbons) represents the critical step. This reaction occurs via a methanol-induced transketolase, dihydroxyacetone synthase (Douma *et al*., 1985; Goodman, 1985; Waites and Quayle, 1983). By a series of rearrangements, xylulose-5-phosphate is subsequently regenerated to form a cyclic pathway; one-third of the glyceraldehyde-3-phosphate molecules generated by the action of dihydroxyacetone synthase enter the central metabolism to become biomass (Babel and Hoffman, 1982). In addition to methanol, short-chain primary alcohols have been used as substrates with reduced efficiency (Tani *et al*., 1972; Sahm and Wagner, 1973; Van Dijken *et al*., 1975, 1976).

5. *The processes used in single cell protein production*.

The processes used in SCP production take into account the different conditions and nutrient requirements needed for microorganisms. Also, these conditions will eventually affect the composition of the protein produced by the microorganism.

In 1948, Olson and Johnson produced a synthetic medium which they found to give yields equal to those obtainable on natural media. They demonstrated that 2-5g/l of asparagine gave high yields of biomass. This stimulation by asparagine was found to increase with increasing sugar concentrations. Mahmoud and Kosikowski (1982), while working on the production of SCP by *Kluyveromyces* in concentrated whey permeates discovered that 1% peptone should be included as an organic nitrogen source to produce the highest biomass yield and lowest alcohol production. The medium they used was composed of 0.3% yeast extract, 0.3% malt extract, 0.5% peptone and 22.5% lactose. A batch cultivation process was used and the fermenter was equipped with an agitation and aeration device, automatic temperature, foam and pH controlling gadgets.

Similarly, maximum yeast growth and more efficient production were obtained by Rolz and de Cabrera (1980) by adding 20.75g of $(NH_4)_2SO_4$, 1.5g of $MgSO_4$ and 3g of H_3PO_4 to one litre of clarified sugar cane juice.

In most cases of SCP production, biomass production by aerobic fermentation was carried out in large fermenters, equipped with temperature, pH and oxygen controlling gadgets (Cooney *et al*., 1980).

A high nucleic acid content is characteristic of rapidly growing cells. The yeasts contain a high amount of nucleic acid, even when expressed on a protein basis (Kihlberg, 1972) The generally accepted safe level of nucleic acid intake in human diet has been shown by Cooney *et al*. (1980) to be equivalent to 20g of yeast. They suggested that a way of reducing the nucleic acid content in single cell protein is

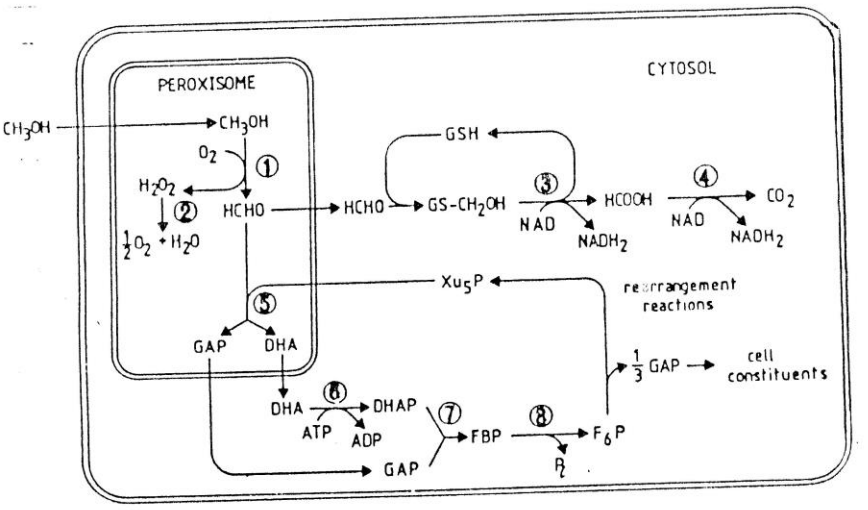

Fig. 1: The methanol pathway and its compartmentalization in *Hansenula polymorphia*. The enzymes catalyzing the various steps are: 1. alcohol oxidase; 2. catalase; 3. formaldehyde dehydrogenase; 4. formate dehydrogenase; 5. dihydroxy-acetone synthase; 6. dihydroxyacetone kinase; 7. fructose 1, 6-bisphosphate aldolase; 8. fructose 1, 6-bisphosphatase.
(Douma *et al.*, 1985).

by using hot 10% sodium chloride extraction which depletes yeasts of nucleic acid. A similar report was made by Kihlberg (1972) who reduced the nucleic acid content of SCP by alkaline hydrolysis of cell ribonucleic acid.

The production of microbial cells on hydrocarbons has undoubtedly been one of the most important developments in industrial microbiology during the past three decades. During the 1960s many large petroleum companies initiated extensive research and development programmes because they were firmly convinced that large-scale production of single cell protein could be designed and engineered without much difficulties. However, they generally underestimated the many difficulties involved in this novel interdisciplinary area. Many basic prblems concerning the biochemistry and physiology of microbial reproduction on hydrocarbons virtually unknown and, as a result, the design of fermentation systems using either crude oils, gas oil, refined alkanes or methane, led to many unforseen complications. It was soon realised that the real costs of production of yeasts on hydrocarbons were not substantially lower than those for yeasts on cheap carbohydrates. the costs on hydrocarbon greatly rose following the "oil politics" of 1973. For the production of yeasts on hydrocarbons, substrates can be either waxy crude oils, gas oils or n-alkanes, usually the C12 to C18 alkanes.

One of the major differences between carbohydrate and hydrocarbon fermentations is the relatively low solubility of hydrocarbons in water. Compared with carbohydrate fermentation, three times the volumetric oxygen is required t grow yeast on hydrocarbons. Thus, for a given production of yeast, the increase in oxygen requirement reduces the output of cells which can be produced per unit time, and this effectivelly reduced the capacity of the fermenter. The difference in oxygen requirements in the production of 1g of dry cells was demonstrated by the following calculation by Peterson (1978):

Carbohydrate $(CH_2O)n$	2.0g
Oxygen	0.34g

compared with

Hydrocarbons $(CH_2)n$	1.0g
Oxygen	0.98g

Thus, hydrocarbon fermentation will produce one-third as much cells (based on O2 limitation) than carbohydrate fermentation, and half as much hydrocarbon as carbohydrate is required for fermentation.

Hydrocarbon fermentation must operate so as to entrain and disperse the oil phase continually. At least a 2-stage continuous fermentation system is required. In the 2-stage system, the first stage serves to optimise cell productivity, the second stage serves to optimise substrate utilization and prepares cells for optimal recovery by minimising the residual oil content and by concentrating the cells. For a second stage, a more traditional type of fermenter could be used.

Hydrocarbon fermentations generate more heat than carbohydrate fermentations. Recovery costs, which are related to concentration and residual oil content of the two cells, as well as cell productivity and substrate utilization costs are also important factors.

Methane is a more difficult medium than liquid hydrocarbons. During methane fermentation, there is a considerable explosion hazard. This can be reduced by carefully adjusting air/methane gas mixture ratios and incorporation of sophisticated control instrumentation as well as the use of fine emulsions and mechanical foam separation.

At the end of the fermentation cycle, the cells in the form of hydrocarbon water emulsions have to be broken in order to achieve maximum cell recovery. Usually, this is carried out in high-speed centrifuges of a nozzle-discharge type. The cells can than be spray-dried.

Wang *et al*. (1980) suggested that filtration without filter-aid is not a successful method for the separation of microorganisms from the media. Instead, multiple centrifugation is the process most often employed. Other alternatives that have been tested due to the differences in sizes amongst microorganisms include foam separation, microfloatation and

flocculation. According to Kihlberg (1972) the fermentation broth initially contains about 1-5% solids. The cell concentration is increased to 10-20% by centrifugation (sometimes complemented by evaporation), and the cream is usually fed to drum or spray dryers to give a dry powder free from viable cells. Tannenbaum and Wang (1975) has suggested that whole yeast cells, or unseparated broken cells, can be processed into texturized products using conventional technology such as extrusion. He employed an extruder to texturise a variety of microorganisms in the form of a cell paste. The paste was heated under shearing agitation, extruded and then pasted through a conditioning zone to give a structured product.

Tannenbaum had earlier in 1967 suggested that harvesting and drying of cells without further treatment may give a product of satisfactory nutritive value but of generally inferior functional qualities. He showed that the value of a protein in food systems actually depends on functional properties such as water absorption, fat-binding, emulsifying and stabilizing ability, film-forming ability, foamability, viscosity, etc. He then concluded that disruption of cell walls increases the possibility of producing protein concentrates or isolates with desirable functional properties. After cell wall disintegration, cell proteins can be precipitated by heat, salt or by pH adjustment.

6. Genetics of single cell protein production.

Yeasts have been used for the production of beer and other alcoholic beverages since the time of Egyptian civilization. The process of fermentation to produce alcohol and a variety of other desirable flavour and aroma has undergone little fundamental changes over the centuries. In contrast, the brewing industry has been subjected to major changes in recent years. New plant and processes have enabled the improvement of production techniques with a concomitant demand for more efficient utilization of raw materials and process plant. Consequently, there has arisen a need to develop new strains of yeasts which are better suited to the requirements of a more advanced, technically demanding, industry. Progress in yeast molecular genetics affords the possibility of satisfying these requirements (Hinchliffe and Vakeria, 1989).

Normal brewing practice results in the production of large quantities of yeast which is surplus to the brewing process. Traditionally, this yeast, often referred to as spent yeast, is sold as a cheap source of biomass to be used in animal or microbial cells or as yeast extracts for human consumption. The brewer obtains an extremely low price for this potentially valuable commodity. Interest has therefore been stimulated in developing alternative uses for this spent yeast which would enhance its intrinsic value. One approach is through genetical manipulation of the yeast to produce high-quality proteins.

Genetic has been suggested as having potential utility not only in reducing process costs, but in improving aspects of process efficiency, developing new strains and improving the quality of spent yeast (Panchal *et al*., 1984); Tubb and Hammond, 1987). This potential is, in most instances, yet to be realised because no strain of genetically manipulated yeast is currently being used in the commercial production of single cell proteins.

The process of production of single cell proteins from yeast involves many stages beginning with the production of yeast inoculum ending in drying of the pellet arising from debittering. The fermentation of the substrate by yeast is crucial to the viability of this process. However, fermentation accounts for less than 25% of the overall operating costs when the raw materials consist of saccharides, especially molasses or cereals (Peterson, 1978). This means that the economic advantage accruing from the application of yeast genetics is limited to a relatively small area of the production process. Consequently, the geneticist is limited to yeast strains used in producing single cell protein which enjoys a large market. Thus, a major objective of any strain improvement strategy is not only the introduction of a new or modified characteristic, but also the retention of existing characteristics. In this

respect, genetic modificaton must have no detrimental effect upon either the cultivation (fermentation) of the yeast or the characteristics they impart to the foods.

Hinchliffe *et al*. (1987) described a process in which heterologous gene expression can be controlled to regulate the synthesis of protein in brewing yeast following beer fermentation. In this respect, a cDNA encoding the human plasma protein, human serum albumin (HSA), was fused to a hybrid promoter formed between the upstream activation sequence of the GAL 1-10 genes and the transcription initiation site of the CYC 1 gene. When introduced into brewing yeast, the resultant CUP 1-bearing plasmid was shown to induce HSA synthesis in the presence of galactose, whereas in the absence of galactose no HSA was produces. This gene regulation system was found to be particularly useful for controlling gene expression in brewing yeast, since induction is very tightly regulated by the presence of galactose in the growth medium. Brewer's wort does not contain galactose. Consequently, it was observed that yeasts harbouring the galactose-regulated HSA gene produced palatable beer, without any detectable synthesis of HSA. Following several successive rounds of beer production, Hinchliffe *et al*. (1987) showed that yeast could be induced with galactose to synthesize substantial quantities of HSA. Thus, the technology has been developed to produce beer with a commercial strain of brewing yeast and to subsequently enhance the value of that yeast by the synthesis of a high-value protein.

Techniques for the genetic manipulation of brewing yeast are now established and are applied to commercial targets. Brewing yeasts are more amenable to recombinant DNA technology than classical genetic techniques (Hinchliffe and Vakeria, 1989). The extent to which the industry embraces new varieties of yeast will depend to a great extent on the perceived acceptability of the strains derived. In this respect, two important aspects must be considered, viz: the yeast must satisfy the commercial constraints imposed, that is, it must be capable of producing desired products. It must also be compatible with the manufacture of food product. However, it appears there is a promising future for genetic manipulation to produce high quality microbial single cell proteins.

7. Concluding Remarks.

Studies on single cell protein production have been carried out extensively in the yeast in the last two to three decades, especially in the areas of cheap raw materials and products which will meet the protein needs of an ever increasing world population. The rising prices of agricultural commodities of all types have already made microbial proteins very attractive because of the advantages that are derivable from producing protein from this source. This trend is certain to continue especially in Nigeria, a developing country, where there is a breakdown of many infrastructures put in place for producing proteins from conventional sources because of economic downturn. Even the continued success of well-planned agricultural programmes will not eliminate the need for supplementary sources of protein that either require no agricultural land at all or utilize vegetable or industrial waste or any other cheap substrates.

Several reports are available showing that the nucleic acid content of SCP sources is high, when compared to conventional food sources. Consumption of such protein sources usually leads to a higher level of plasma uric acid and an increased excretion of uric acid in the urine. Various methods have been described for the reduction of nucleic acid in SCP. The manufacturers of SCP can choose the process that not only yields low amounts of nucleic acid, but also one that integrates this technology with processing parameters and possibly controls or alters the flavour and functionality of SCP.

Recenet research efforts have been directed towards genetic engineering of organisms for SCP production. Mutants of brewing yeasts have been developed which produce high quality beer in addition to the synthesis of high value protein.

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