CHAPTER- I

1. INTRODUCTION
2. LITERATURE SURVEY
3. PRESENT WORK AND ITS SCOPE

"To Know that we know what we know
and to know that we don't know what we don't know
is true knowledge". - Confucius - Chinese Philosopher.

I. INTRODUCTION

Paraffinic hydrocarbons encompass the whole spectrum of saturated hydrocarbons from methane upto about tetracontane.

At present natural gas and petroleum are still available in large amount for the production of paraffinic hydrocarbons.

Petroleum is composed essentially of four distinct types of hydrocarbons which differ from one another in chemical structure. They are : Paraffins, Olefins, Naphthenes and Aromatics.

From both natural gas and petroleum, low molecular weight normally gaseous or very low boiling paraffinic hydrocarbons such as methane, propane, the butanes and the pentanes can be isolated in the individual state. But petroleum also yields the higher molecular weight representatives of the type paraffinic wax and microcrystalline wax - that is hydrocarbons mixture with about 20 to 25 carbons atoms and more. The intermediate

molecular sizes with 10 - 20 carbons atoms, from decane to eicosane could not until recently be obtained with high purity.

Straight chain paraffinic hydrocarbons of 10 - 30 carbons atoms could be separated from petroleum fractions selectively by the so called extractive crystalline process by means of urea and several commercial units are in operation. (1, 2, 3). Sonnebonn's Petrollia Refinery Pennyslavania in operation since 1950 is said to be the first commercial unit applying urea dewaxing. (4, 5).

In recent years adsorption processes have been developed by which a far reaching removal of n. paraffins can be obtained. In these processes the n. paraffinic hydrocarbons are adsorbed on the adsorbent while branched paraffinic hydrocarbons and aromatic hydrocarbons together with cyclic paraffins pass through. Such adsorbents are called molecular sieves. Using sieves, unaffected by sulphur, water, olefins nitrogen or trace metals, normal paraffins of 99 Wt % purity can be recovered from a typical Kerosene stream. (6). Considerable attention

has been given to the production of n. paraffins in the C_{10} - C_{14} range for use in the manufacturing of linear Alkyl Benzene Sulphonate (LABS) detergents. (7, 8).

The paraffin waxes were produced by pressing paraffin distillates and sweating the resulting slack wax for final removal of oil. As swaeting is applicable only to waxes of relatively low melting point and low molecular weight all the waxes so produced were of similar characteristics i.e. they formed large crystals and were relatively hard, of low viscosity and confined to a melting range of about 50 to 60°C. Microcrystalline waxes on the other hand were obtained by solvent crystallization methods from wax distillation residue. Many now prefer in accordance with the above to designate distillate of low molecular weight waxes as paraffins and residual high molecular weight waxes as microcrystalline. Because the original paraffins waxes relatively high in straight chain hydrocarbons (as indicated for example by adduction with Urea) normal paraffin content has been used as a means of distinction. Many of the proposed definition emphasized that paraffins contained relatively high percentage of

normal or slightly branched alkanes, where as microcrystalline waxes contained substantial proportions of highly branched or cyclic hydrocarbons. Chemically both paraffin and microcrystalline waxes consist of saturated hydrocarbons.

Paraffins and microcrystalline waxes are both long chain compounds, but microcrystalline waxes have much higher molecular weight. The common commercial paraffin wax have molecular weight of 360 - 420 which means that the average molecule in these hydrocarbons contain 26 - 30 carbon atoms. The molecular weight of commercial microcrystalline wax is 580 - 700, that is average molecule contains 41 - 50 carbon atoms.

The hydrocarbons fraction from petroleum are of non uniform composition and the ratio of the individual types of compounds such as paraffins, naphthenes and aromatic compounds in the mixture is fundamentally dependent on the origin of the crude oil.

The gaseous reaction production of the hydrocarbons of coal lignite and tar are further sources of lower paraffins.

For the utilization of paraffinic hydrocarbons, processes developed could be broadly classified as under :

(i) Oxidation

(ii) Chlorination

(iii) Nitration

(iv) Sulphochlorination & Sulphoxidation

(v) Fermentation

Alcohols, Aldehydes and fatty acids are some of the products studied by the oxidation of paraffinic hydrocarbons. The manufacture of soap fatty acids has attracted attention of industrialists.

The chlorination reaction is the oldest substitution reaction of the paraffins. The chlorination of paraffins lead to intermediate product for the synthesis of detergents, textile auxiliaries lubricating oil, pour point depressants leather oil etc.

The most important use of nitro paraffins is undoubtedly in the field of solvents and intermediate product.

Similarly the product of sulphochlorination, sulphoxidation are mainly used in detergent industry.

Utilization of paraffinic hydrocarbons for the production of Single Cell Proteins is of recent origin and is achieved through fermentation which today refers to both aerobic and unaerobic metabolic activities of microorganisms.

Necessity is the mother of invention. In order to overcome their dependence on America and to stop importing soya bean mainly used in Animal compound feed industry as the source of protein, rich oil companies in Europe, got involved during early sixties in research and development activities to produce Single Cell Proteins by cultivating certain microorganisms on hydrocarbons present in Petroleum fractions. 1973 oil crisis and due to political pressure they has to abandon their commercial plans. However ICI Ltd. based on their knowhow developed, cultivating Bacterial strain on Methanol as carbon substrate, set up 70000 tonnes per annum, plant in Birmingham and their product is marketed in Europe under the trade name " Pruteen ".

In India unlike in Europe the population is still growing at 2% level. Green and White revolution though brought some relief in meeting the demands of cereal and milk, natural calamities such as drought or heavy floods, cause considerable damage and it is long way to go to provide nutritious and well balanced food to the hungry population. Animal compound feed industry, though it is in infant stage, has realized the importance of feeding animals with protein enriched formulation to increase milk as well as egg yield, and groundnut cake and fish protein concentrate are in great demand. India being a fast developing country needs to strengthen its foreign exchange reserves and groundnut cake is a potential earner of foreign exchange.

Single Cell Protein attracts the attention of scientists, due to its high nutritive value compared to vegetable proteins and its production is not inhibited by climatic conditions. Yeast and Bacteria are unicellular fast growing microorganisms and they have been found to grow on Methanol, Ethanol, N. Paraffinic hydrocarbons

present in Kerosene and diesel fractions, Whey, Sulphite waste liquor, Molasses, Cellulose and Starch materials.

Today around 90% of the hydrocarbons present in Petroleum crude oil is being used for power generation and its utilization for providing better nutritious food is a welcome proposition.

I. 2. UTILIZATION OF N. PARAFFINIC HYDROCARBONS :

Today there are six different processes which can be carried out industrially for the utilization of paraffinic hydrocarbons :

1. Oxidation
2. Chlorination
3. Nitration
4. Sulphochlorination
5. Sulphoxidation and
6. Fermentation.

I. 2. 1. Oxidation of paraffinic hydrocarbons :-

Oxidation of paraffins has been operated industrially, in the first place with paraffin wax, in order to obtain fatty acids from the mixture of hydrocarbons with 20 - 25 carbon atoms. In the oxidation of paraffins, fatty acids of various molecular weight are obtained through the breaking of carbon chains. The crude fatty acid mixtures are separated by distillation into three large fractions and a residue :

- Forerun fatty acids with 5 - 11 carbon atoms.

- Soap fatty acids with 12 - 18 carbon atoms.
- Residual fatty acids with more than 18 carbon atoms.

The soap fatty acids can in all cases successfully replace the higher saturated fatty acids of animal and vegetable fats.

At an early stage the unavoidable formation of forerun fatty acids raised doubts about the economic feasibility for the oxidation of paraffins, since it was at first not known how to utilize them. Today however, they are much sought after since they can be converted by catalytic hydrogenation into primary alcohols which are important constituents of plasticizers.

Much has been written on the oxidation of the lower paraffinic hydrocarbons and there are innumerable patent specification dealing with the technical side of the oxidation processes, particularly with respect to the manufacture of oxygen containing compounds such as formaldehyde, acetaldehyde and acetic acid. On working with

gaseous paraffinic hydrocarbons it is important to know the explosion limit.

When methane is used only three oxidation products can arise namely methanol, formaldehyde and formic acid. In the oxidation of propane or butane large number of oxygen containing compounds are formed. Usually it is not pure methane which is used but natural gas in which, as is well known, ethane and propane are also present.

Frolich and Wiexivich (9) found that the addition of ethane to pure methane markedly increased the yield of methanol to about twice that which could arise if all the ethane were converted into methanol. Bibb and Lucas (10) oxidized methane, containing 70% ethane, at atmosphere pressure at 700 - 750°C in the presence of small amounts of nitrogen dioxide and obtained methanol and formaldehyde in an 8 : 1 ratio.

The oxidation of ethane at a pressure of 100 atm and 260 - 270°C gives ethanol and methanol in 2 : 1 ratio (9).

The oxidation of propane at 170 atm. and 350°C using a mixture of propane and oxygen containing 7 - 9% of oxygen yield methanol, ethanol, propanol, acetaldehyde, formaldehyde, acetone, acetic acid and formic acid (9). The same authors studied the oxidation of pentane mixtures, containing 60% pentane and 40% isopentane at 135 atm. and temperatures 300 - 500°C and it was found to give small amount of methanol and formaldehyde and large amounts of acetaldehyde, methyl ethyl ketone and propyl alcohols and secondary amyl alcohols.

The oxidation of higher paraffinic hydrocarbons such as heptane has been investigated by Widmaier and Mauss (11). At 143 - 173°C and pressures up to 6 to 7 atm. peroxides are first formed which then decompose to form aldehydes for example acetaldehyde, propionaldehyde and butyraldehyde apart from other materials.

The conclusion which can be drawn from the various published experimental results are :

- The higher the pressure in oxidation the greater is the

yield of alcohols and the lower the yield of aldehydes and acids.

- A high velocity of flow of the reacting gases favours the formation of higher alcohols and lower velocities of flow lead to the formation of lower alcohols and acids, the formation of water and carbon dioxide also being favoured.
- An increase in the temperature has much the same effect.

Cities Services Oil Co. at Tallant Oklahoma was the first firm to undertake the industrial oxidation of paraffinic hydrocarbons (12). Another large scale plant for the oxidation of paraffinic hydrocarbons has been built by the Celanese Chemical Corp. at Bishop in Texas (13).

Another type of oxidation of gaseous paraffinic hydrocarbons namely that using solvents in the presence of catalysts is operated by Celanese at Pampa in Texas (14). Here butane in acetic acid solution with the addition of cobalt or manganese salts is oxidized principally to acetic acid. In addition acetaldehyde, acetone, propionic

acid and methanol are produced. In Germany two processes have been developed - the Gutehoffuungshutte process and Hibernia process to obtain formaldehyde from the methane of coke oven or hydrogenation gases by oxidation.

The process for the preparation of carbon monoxide hydrogen mixture as the starting material for the large scale operation of the Fischer - Tropsch synthesis by the partial oxidation of natural gases (methane) plays a very large role.

Auto thermic methods are used in the manufacture of ethylene from ethane or propane and the production of acetylene by the Sachsse process (15, 16, 17).

The Production of fatty acids by the oxidation of paraffin wax with regards to the manufacture of " Soap fatty acids " to economic fats suitable for human nutrition was studied in detail by Reichenbach, Holstadter (18), Wiilick (19), Gill and Meusel (20), Bolly (21), Engler Bock (22) and Schaal (23).

In 1921 the Badische Anilin and sodafabrik took up

the problem of paraffin wax and several tonnes of synthetic fatty acids were manufactured. In 1948 a large plant with a capacity of about 20,000 tonnes was constructed at the Deutsches Hydrierwerk at Rodleben. Other than American it is mainly Soviet chemists who have investigated the oxidation problem. In the Soviet Union, synthetic fatty acids are manufactured for industrial purposes on a large scale at Shebekino in the Belgorod region, by the oxidation of paraffin wax. While in America and the Soviet Union paraffins wax from petroleum has been the main starting material, in Germany lignite paraffins wax oxidized mainly until pure synthetic paraffins wax became available through the Fishcher Tropsch Ruhr chemic process.

The paraffinic starting material obtained from the medium pressure synthesis offers a great advantage for the oxidation process in that it contains a substantially greater proportion of straight chains than the product obtained under pressure. It therefore gives fatty acids containing a smaller amount of branched components. This is important since the undesired, often pungent smell of the synthetic soaps is due mainly to branched fatty acids.

I. 2. 2. Chlorination of paraffinic hydrocarbons :

The chlorination reaction is undoubtedly the oldest substitution reaction of the paraffins.

Chlorination products of methane play an important role as solvents, those of ethyl chloride in the preparation of tetraethyl lead. The chlorination of the higher paraffins lead to intermediate products for the synthesis of detergents and textile auxiliaries, lubricating oils, pour point depressants, leather oil etc.

Chlorination as such can in general be carried out in three different ways :

1. Photo Chemically
2. Catalytically
3. Thermally

Chlorination reaction is highly exothermic and it is assumed about 24 K cal/mole is liberated in this substitution reaction. In technical applications it is assumed that about 30 K cal is liberated per kg. of chlorine reacted.

Photo chemical chlorination :-

The primary process consists in the splitting of a chlorine molecule by the adsorbed light energy to give chlorine atoms. A chlorine atom now reacts with a hydrogen molecule to form hydrogen chloride and a hydrogen atom. The hydrogen atom in turn reacts with a chlorine molecule to give hydrogen chloride and liberates a chlorine atom. In this way the chlorine atom formed originally by means of the light energy is reformed in another manner and can continually induce new reactions. However chain reaction are not unrestricted in their lengths but the so called chain termination occurs sooner or later, according to the particular reaction in question. In the case of chlorination chain termination occurs only through recombination of chlorine atoms to form chlorine molecules, which takes place on the walls of the vessel and through so called chain breakers - impurities which bind both chlorine and hydrogen atoms so that these are eliminated for further formation of hydrogen chloride molecules. In the photo chemical chlorination of the paraffinic hydrocarbons quite similar chain reaction occur. The quantam

yield in the chlorination of n.heptane in the presence of ultra violet light given by Stauff and Schumacher (25) is 7,000. It has been calculated that the action of ultra violet light on chlorine produces the same affect as heating to 1500 °C. The chlorination of methane up to carbon tetra chloride stage has always claimed particular attention while the photo chlorination of n. butane has also been developed.

A thorough discussion of technical photo chemical process, the construction of reactor systems, energy costs, reaction mechanism has been given by Doede and Walker (26). A high pressure reactor for the photo chemical chlorination and sulphochlorination has been described by Boynton et al (27).

Catalytic Chlorination :-

Catalytic chlorination can be used both with gaseous and liquid hydrocarbons. If it is desired to chlorinate gaseous hydrocarbons catalytically in a condensed system, carbon tetra chloride is preferably used as solvent.

Thermal Chlorination :-

Thermal chlorination takes place in the absence of catalyst and light and is used particularly for the lower paraffins. The energy of activation in thermal chlorination is 20,000 cal/mole while in catalytic chlorination it is only 12,000 cal/mole.

Rust and Vaughan (28) studied the complexity of the reaction sequences and have thrown much light on the conditions of thermal chlorination. Special processes have been developed for the high temperature chlorination of methane, propane, n. butane and isobutane. Hass (29) has described laboratory apparatus for the thermal chlorination of low molecular weight normal gaseous hydrocarbons.

Chlorination of higher molecular weight paraffinic hydrocarbons are often carried out either for the preparation of a component for the manufacture of synthetic lubricating oils or for textile auxiliaries and detergents.

The chemical utilization of the chlorination products of the paraffinic hydrocarbons :-

A great variety of intermediate and finished products

in the field of aliphatics have been successfully obtained from the alkyl chlorides :

- Hydrolysis to alcohols.
- Reaction with alkali metal cyanides to give nitriles which can be converted into fatty acids by saponifaction and also into amines by reduction.
- Reaction with sulphites to give water soluble sulphonates.
- The formation of Grignard compounds and the reaction of these with carbon dioxide to give carboxylic acids.
- Reaction with anhydrous sodium acetate to give esters.
- Conversion into thioalcohols by reaction with alkali metal hydrogen sulphides.
- Friedel - Crafts reaction with aromatic hydrocarbons.
- Dehydro chlorination to olefins.
- The introduction of alkyl groups, particularly higher alkyl group into pharmaceuticals, pesticides and insecticides.
- Aminolysis to amines.

The Utilization of the chlorinated product of the lower hydrocarbons :

The direct chlorination of methane is used today primarly to prepare methyl chloride which is used widely as solvent.

- Methyl chloride has been very widely introduced as a refrigerent for small industrial refrigerating plants.
- Very large amounts of methyl chloride are used as solvents for the preparation of butyl rubber by the copolymerization of isobutylens and 2 - 3 percent of isoprene or butadiene.

In USA sixty percent of the production of methyl chloride is used for silicones, thirty percent for butyl rubber and the remaining ten percent for small scale manufactures, e.g. cellulose methyl ether, pesticides etc.

Methylene chloride which is available through the chlorination of methane or methyl chloride is an excellent solvent for fats, oils and resins. It can also be used for example in association with butylalcohol for dewaxing

of lubricating oils, since at low temperatures, it dissolves little wax but all the oil. Together with benzene it is particularly suitable for the extraction of fats and oils from seeds, of lecithin from soya beans, and of cocoa butter from cocoa beans. It is also used with advantage in the paint and varnish industry.

Chloroform which can be prepared not only by the direct chlorination of methane or its monochloro and dichloro derivatives is a mobile liquid of outstanding solvent power for fats oils resins rubber etc. Its use as anaesthetic is very wellknown. In the United States and Canada about 13% of chloroform is added to carbon tetra chloride to give a frost resistant fire extinguishing agent for air craft with a freezing point of -50°C.

The action of hydrogen fluoride on chloroform in the presence of antimony fluoride yields chlorodifluoro methane (Freon 22).

Freons is registered trade mark for lower paraffinic hydrocarbons containing chloride and fluoride, in England

difluorodichloro methane is called ARCTON - 6 and difluoromonochloro methane is called ARCTON - 4 and monofluorotrichloro methane is called ARCTON - 9.

The lower molecular weight chlorofluro methane and ethanes are being used more and more as aerosol propellent.

The propellants were first used for spraying insecticides and consisted partly of liquid propane or butane in which the active materials were dissolved. Carbon tetra chloride is also widely used solvent for organic materials. It is used on large scale for the cleaning of textiles in laundries and other cleaning establishments.

Tetra ethyl lead is produced in very large quantities since it raises the octane number of gasolines with very poor antiknocking properties, very considerably. This was discovered in 1852 by Bowig. In 1921 Midgley and Boyl (30) in the research laboratory of the General Motor Corporation found that it has a favourable effect on the knocking characteristic of poor gasolines. It was found later that when leaded gasolines was used lead and

lead dioxide deposited on the pistons and valves of the motor. However the addition of ethylene bromide, which converts the lead into the comparatively volatile lead bromide which is removed with the exhaust gases, has proved to be the best. This mixture is marketed under the name Ethyl fluid (31).

The chlorination products of propane are not of great importance. Mono chlorination product of butane were obtained industrially for the manufacture of butylene which could be of use as starting material for the manufacture of fuel with good antiknock properties.

1, 2 dichloro butane may be obtained by the direct chlorination of butane and Butadiene could be obtained from 1, 2 dichloro butane at 500 - 550°C with an 85% yield (32).

An interesting possibility for obtaining carboxylic acids consists in the action of metallic sodium and carbon dioxide on alkyl chloride under pressure in the presence of a solvent (33).

Utilization of the chlorination products of the higher paraffins :

A particular applications of the product of the direct chlorination of the higher hydrocarbon consists in their conversion into lubricating oil additives, pour point depressants, leather oil and detergents and textile auxiliaries. Valuable synthetic lubricating oils especially oils with a high viscosity index can be obtained from chloro-paraffins.

The earlier expectation that valuable cationic detergents and textile auxiliaries which would help to reduce the consumption of vegetable and animal fats could be produced by the treatment of chlorination products of petroleum or its fraction with ammonia, has not been realized in a technically satisfactory manner.

Paraffins wax such as is available in large amounts and with adequate purity from petroleum, or from Fisher Tropsch synthesis or from lignite low temperature carbonization tar, after melting and treatment with a catalyst such as iodine or antimony penta chloride or even without

a catalyst, but then at a somewhat higher temperature is chlorinated by the introduction of gaseous chloride until at least one chlorine atom has been uptaken per molecule of paraffins.

The chloroparaffins so obtained can in general be converted into a synthetic lubricating material in three ways.

- The chloroparaffins can be caused to react with aromatic hydrocarbons, such as xylene, naphthelene etc., with anhydrous aluminium chloride in a Fridel Craft Process, one or more alkyl groups entering the aromatic nucleus according to the process condition.
- The chloroparaffins is polymerized directly to lubricating material by the action of anhydrous aluminium chloride or activated aluminium with the liberation of hydrogen chloride.
- The chloroparaffins is converted catalytically over a solid catalyst into hydrogen chloride and olefins and the resulting olefins are polymerized by themselves by means of aluminium chloride.

There are several possibilities for the industrial use of the chlorination product of the higher paraffins. They can be used directly as substitution for tallow in the leather industry, as lard oils in the form of emulsion in the textile industry, as substitution for linseed oil and castor-oil in the paint and varnish industry as plasticizers for polyvinyl compounds, for the preparation of flame proof impregnating agents for paper and textile ror high pressure lubricating oils etc (34).

Chlorinated paraffin wax :-

There are roughly three main types of chlorinated paraffins wax prepared technically (35).

1. A chlorinated wax containing about 43% chlorine corresponding approximately to 7 atoms of chlorine in a paraffin molecule with 25 carbon atoms. This is yellow non volatile liquid.
2. A chlorinated paraffin wax containing 60% of chlorine (about 15 atoms of chlorine per C_{25} mole) with a Melting point of about 50°C and

3. One with a chlorine content of about 70% corresponding to 22 atoms of chlorine per C_{25} molecule. This is solid and brittle and softens at 90 - 100°C.

The highly chlorinated wax are used in the United States for various purposes under the name chloro wax. Chloro wax 70 a paraffin wax containing 70% chlorine is inert chemically it does not condense or polymerize and is stable to acids and even to dilute alkali. It is used chiefly for impregnation of and the preparation of flame proof textiles and paper. Wooden roof tiles may also be impregnated with chlorinated paraffin waxes and are fire proof. Chloro wax 40 is suitable as a plasticizer for vinyl polymers and also for ethyl cellulose. The chlorinated paraffins waxes are used not only as plasticizer or extenders e.g. in poly vinyl chloride but also used in high pressure lubricating oils and cutting oils (36). Further their addition to polyethylene can reduce the flammability of the latter.

Chlorinated paraffin wax can be mixed well with castor-oil in any proportion. They act as solubilizers

between castor-oil and mineral oil which are not otherwise miscible.

II. 1. 3. Nitration and Nitration products of the paraffins :

Three industrial nitration processes of paraffinic hydrocarbons are used :-

- gas phase nitration process for the lower member upto hexane
- liquid phase processes working without pressure which can be used only for hydrocarbons having a boiling point of 180°C.
- liquid phase pressure process.

The most important application of the lower nitroparaffins is undoubtedly in the field of solvents and intermediate products.

In a series of investigations Degering and Co-workers have dealt with the possibilities of the chemical utilization of the lower nitroparaffins and have studied

previously known reaction in more detail and extended their range of application.

First communication - the production of amines (37).

Second communication - the production of oximes (37)

Third communication - the production of nitro alcohols from aldehydes containing no other functional groups (38).

Fourth communication - Nitro diols (Nitroglycols) from simple aldehydes (39).

Fifth communication - Reduction of nitroalcohols and nitroglycols to the corresponding Amines (40).

Sixth communication - Production of aldehydes and ketones from nitroparaffins (41).

The most important reaction of the lower nitro paraffins, from a technical point of view, so far, is their reaction with aldehydes and ketones specially with formaldehyde to give nitroalcohols.

I. 1. 4. The Sulphochlorination and Sulphochlorination Products :

By sulphochlorination is understood the combined reaction of sulphur dioxide and chlorine with saturated aliphatic hydrocarbons under the action of ultra violet light.

The direct sulphonation of the paraffinic hydrocarbon with sulphuric acid, oleum or sulphur trioxide is still an unsolved problem. In the aromatic series the reaction takes place very smoothly. In the case of paraffins the reaction is not possible to the same extent because of the insolubility of the sulphonating agent in the hydrocarbons and because of the ready thermal decomposition of the alkane sulphonic acids. Consequently the discovery was more important, that, by the combined action of sulphurdioxide and chlorine on liquid or gaseous hydrocarbons under certain condition, which can be easily achieved, aliphatic sulphonyl chlorides are formed which can be converted into sulphonic acids and their salts.

Because of the very great mobility of the chlorine atoms bound to the sulphur the sulphonyl chlorides possess a high reactivity which makes them capable of undergoing numerous reactions.

Sulpho chlorination is therefore a pioneering reaction in the chemical utilization of paraffinic hydrocarbons by substitution processes and is a distinctive example of the fact that the inertness of the paraffins is not a general phenomenon.

The actual composition of the end products produced by the combined action of sulphur dioxide and chlorine on paraffinic hydrocarbon was determined accurately by German and American investigators independently. They were able to show that these products are exclusively sulphonyl chloride and sulphochlorination takes place without the formation of any other sulphur containing intermediate products. Sulphochlorination products are quite stable to acid hydrolysis and by prolonged boiling with water they are converted slowly into hydrogen chloride and sulphonic acids.

After the sulphochlorination reaction has been carried out the reaction mixture contains the following products :

- Mono sulphonyl chlorides
- Di & polysulphonyl chlorides
- Chlorinated mono sulphonyl chlorides
- Chlorinated di & polysulphonyl chlorides
- Alkyl chlorides
- Unattacked starting material.

When the lower paraffinic hydrocarbons are sulphochlorinated the individual reaction products can be separated by distillation. The di and polysulphonyl chlorides and then chlorinated derivatives remain in the residue. The higher aliphatic sulphonyl chlorides can no longer be distilled even under a high vacuum, since on treating to temperature above 100°C they are converted into alkyl chlorides with the loss of sulphur dioxide.

The very great reactivity of the hydrolysable chlorine bound sulphur, makes the aliphatic sulphonyl

chlorides capable of the most diverse reaction. Some of the most important reaction of sulphonyl chloride are :

1) Saponification of sulphonyl chlorides with alkali hydroxide solution. The alkane sulphonate with sufficiently long paraffinic residues (C_{12} - C_{20}) have excellent surface active and detergent properties.

2) The reaction of the aliphatic sulphonyl chlorides with phenols gives compounds insoluble in water. The water insoluble substances are esters of alkane sulphonic acids. These oils have an excellent capacity for dissolving various plastics, they dissolve P.V.C. as well.

The water soluble or alkali soluble reaction products of the higher sulphonyl chlorides with phenols or naphthols containing sulphonic or carboxylic acid groups can be used as emulsifiers or soap substitutes.

3) The reaction of paraffinic sulphonyl chlorides with alcohols and thio alcohols : Aliphatic sulphonyl chlorides with lower alcohols such as methanol, ethanol etc. results in the formation of alkyl chlorides and sulphonic acid.

Depending on their chain lengths, the sulphonyl chlorides themselves form insecticides, tanning agents and high pressure lubricants.

The higher sulphonates which are well known as detergents under the trade name " Mersolats " are also used successfully for the manufacture of toothpastes, shampoos, mouth wash,disinfectants.

The most important application of the sulphonyl chlorides of the higher molecular weight paraffins is undoubtedly in the field of detergents.

I. 2. 5. The Sulphoxidation and other Substitution Reactions :

By sulphoxidation is understood the combined action of sulphur dioxide and oxygen on saturated aliphatic hydrocarbons in the presence of ultra violet light. This reaction was first discovered in 1940 (42). Although the paraffins can not be sulphonated in the same way as aromatics, which can be converted into sulphuric acids by the direct action of concentrated sulphonic acid or oleum,

sulphoxidation prowide a process which permits aliphatic sulphonic acids to be made in a simple way.

The sulphonic acids of the lower, normally gaseous, paraffinic hydrocarbons from methane to pentane and also those with 5 - 10 carbon atoms have so far not been used in the chemical industry to a great extent. On the other hand the sodium salts of monosulphonic acid from decane to eicosane exhibit considerable activity in washing foaming, emulsirying, wetting and floatation agents etc.

From the industrial point of view of sulphoxidation reaction is mainly of interest for the conversion of paraffinic hydrocarbons with 10 - 20 carbons atoms into detergents. Only saturated hydrocarbons are capable of undergoing sulphoxidation.

In addition to chlorination nitration, sulphochlorination and sulphoxidation other substitution reaction of the paraffins hydrocarbons are chlorophosphonation, carbochlorination, cyanation, nitrosachlorination and nitrosation.

Utilization of paraffinic hydrocarbons for the production of Single Cell Pfoteins by fermentation is of recent origin.

I. 2. 6. Fermentation :

One of the earliest reports concerning the utilization of hydrocarbons by microorganisms was published by Miyoshi (43). He observed that the fungus Botrytis cinerea could attack paraffin. However the first reference to a yeast utilizing hydrocarbon was made by Perrier in 1913. During the second world war a series of fundemental papers appeared on the developing area of petroleum microbiology. Zobell, Just, Bushnell et al were the first to grow Candida tropicalis and Candida lipolytica under non sterile conditions and without infections (44, 45, 46, 47). A further report, that Candida tropicalis grew on paraffins, was made by Hoerburger (49). Later more general reviews on hydrocarbon microbiology appeared (50, 51, 52, 53, 54, 55, 56, 57, 58).

The extensive studies carried out in an attempt to

utilize alkanes as carbon sources for the production of a variety of useful compounds such as cell mass, proteins, carbohydrates, nucleic acids, lipids, amino acids, organic acids, vitamins and coenzymes or antibiotics were reviewed by Fukui and Tanaka (57).

From biochemical point of view these were divided into three groups. The first group involves products common to those of conventional microbial processes using carbohydrates as substrates. These include Amino acids, Organic acids, Carbohydrates, Nucleic acids, Nucleotides, Nucleosides, Vitamins and coenzymes, Antibiotics and Cell mass. Alkane substrates being hydrophobic in nature favour the production of water insoluble compounds. These products belongings to the second group are also common to carbohydrate processes, but their productivity is enhanced markedly by special features in alkane assimilation. They include Trehalose lipids, Rhamno lipids, Lipopoly saccharides, Ribo Flavin, Cytochrome C, Coenzymes Q, Ergosterol, Catalase, Uricase lipoproteins, Biotin vitamer etc. The third group includes products which

have structures closely related to alkane substrate and can therefore, be specifically produced in alkane media. Methylcitrate, Methylosocitrate, Monocarboxylic acids, Dicarboxylic acids, Fatty alcohols enzymes participating in oxidation and degradation of alkanes are some of the products under this group.

The lower homologous of the paraffin series, which are liquids at room temperature (n. pentane, n. hexane, n. heptane, n. octane and n. nonane) are usually not capable to being utilized for growth by microorganisms. It seems that this is due to their ability to dissolve cell membranes or membrane bound protein structures, especially those associated with the transport and oxidation of these substrates. Pure liquid n. alkanes are not produced on a commercial scale, but mixture are available.

Using pure n. paraffins as substrate the bioprocessing can be adjusted to consume almost 100 percent of the added feed stock (Yielding about 1 kg of biomass and 1.6 kg of carbon dioxide and by-products for each kg of n. paraffins consumed). In this way the residual hydrocarbon

content of the effluent is reduced to a minimal level and a fully acceptable product can be obtained by a simple concentration and drying process.

Normal paraffins containing from fourteen to twenty carbon atoms are the most suitable carbon source for yeasts. If n. alkanes are used which have melting point higher than the growth temperature, they will be present as a solid suspension in the aqueous medium. If these high molecular weight paraffin can be maintained in a molecularly labile state by dissolving them in a (microbiologically) inert liquid hydrocarbon phase, or by using a mixture of n. alkanes of a carbon number range that is liquid at growth temperatures, growth comparable with that of the liquid n. alkanes can be obtained. Although mixtures of pure normal paraffins are the preffered substrates for the growth of microorganisms, due to lower costs and ease of processing, semi crude cuts of petroleum containing a percentage of n. alkanes can be used with success.

Kerosene varies in composition according to the

source of the crude petroleum, the method of distillation, the boiling range of its component hydrocarbons and subsequent processing. A typical crude kerosine from a Middle East Oil may have a boiling range of approximately 180°C to 240°C and contain about 30 percent n-alkanes, 20 percent iso-alkanes, 30 percent cycloalkanes and 20 percent aromatics, principally in the range of C_{10} to C_{16}. Hydrotreating carried cut on premium grade kerosine, reduces the content of aromatics. Gas oil meaning diesel oil is a heavier petroleum out than kerosine. From a representative Middle East Crude, a light gas oil would have a boiling range in the region of 230 - 290°C and contain 20 - 25 percent n-alkanes, 25 - 30 percent iso-alkanes, 20 - 30 percent cyclo-alkanes and about 20 percent aromatics.

A heavy gas oil e.g. 290 - 340°C could have a continuing trend to lower n-alkane content with increasing boiling point, containing upto 20 percent n-alkanes, 25 percent iso-alkanes, 20 percent cyclo-alkanes and 35 percent aromatics. The carbon number range of hydrocarbon in gas oil would be mainly between C_{15} and C_{22}.

Zobell (59) formulated four rules for the specificity of utilization of hydrocarbons by yeasts. Shennan and Levi (53) due to their own discoveries modified Zobell's formulation.

Zobell's rule 1 : Aliphatic compounds are more readily attacked than aromatic compounds. Shennan and Levi (53) modified this rule to : Aliphatic hydrocarbons are assimilated by all strains of some yeasts in many genera, other classes of compounds including aromatics may be oxidized but are not usually assimilated efficiently.

Zobell's rule 2 : Long chains are degraded preferentially to short chains. The revised form of this rule is n-alkanes of chain length shorter than n. nonane are not usually assimilated by yeast, but may be oxidized.

Zobell's rule 3 : Unsaturated compounds are degraded more readily than saturated compounds. Yeast may utilize 1 - alkenes as growth substrates but the yield is usually much lower than that obtained with the corresponding saturated n-alkene.

Zobell's rule 4 : Branched chain alkenes are degraded more readily than unbranched molecule. Shennan and Levi (53) modified this to : Branched chain compounds are degraded less readily than straight chain compounds.

I. 2. 6. PARAFFIN AS FEED STOCK IN THE FOOD FRONT

I. 2. 6. 1. Role of Proteins in Economic Development :

On the adoption of n. paraffinic hydrocarbons as a new feed stock in the world economy, the role these native constituents of petroleum can play in supporting protein needs of growing demand in the world and then in India is analysed. The global demand for food protein is rapidly increasing with rapid increase in world population and increase in incomes in the developing countries.

Food and agricultural organization (FAO) of the United Nations, estimates during the period 1980 to 1990 the annual rate of increase to be 1.8% and for the period 1990 to 2000 it will be 1.6%, the population in 1990 amounting to 5.16 billions and the same in 2000 amounting to 6.08 billions.

Similarly the economic growth of the world has been estimated. The annual rate of increase of total Gross Domestic Product (GDP) of the world at the 1975 price was 4.12 percent during the period 1975-85 and is likely to be reduced to 3.34 percent during the period 1985-2000.

The annual rates of increase of GDP during respective period in developed countries is 3.87 percent and 3.05 percent and those of developing countries is 5.00 percent and 4.27 percent respectively (60). Even in regional assessment developing regions surpass developed regions. However in terms of GDP per year per capita developing regions fall below developed regions because of the presence of still continuing population growth.

The growth of population causes the proportional increase in required amount of food and the increased GDP also increase the food demand in terms of both quantity and quality. Generally protein food stuffs are more expensive than calorie food stuff and in particular animal protein are equivalents to the quantitative side of protein. Thus there exist a close correlation between protein supply per day per capita and economic power. Krishna has made an exhaustive study on the socio-economic situation in developing countries and potential for the production of protein in his report to UNIDO. (61).

The world supply of total proteins in 1975 was 100 million tonnes and the demand rose to 128 million tonnes in 1985 and is expected to reach the potential demand of 178 million tonnes in 2000. The annual rate of increase is 2.3 percent during the period from 1975 to 2000. The total demand per day per capita increases from 68 gms in 1975, through 72 gms in 1985 to 76.8 gms in 2000, the annual rate of increase being 0.4%. While total protein intake increases gradually, animal protein intake in the world increases markedly by 2.3 times from 35.4 to 81.0 million tonnes at the annual rate of increase of 3.4 percent during the period from 1975 to 2000 (59).

Most of the countries in Europe could restrict, their population growth and attain zero growth level. The understanding of nutritional requirements of different animals has led to the growth of animal feed compounding industry with tremendous commercial implication. In the nine countries following the European Economic Community (EEC) compound animal feed production has risen from 22 million tonnes in 1960 to about 60 million tonnes in 1975 and in the case of Japan, during the same period, it has risen

from 2.0 million tonnes to over 20 million tonnes, registering an annual increase of nearly 4 to 5 percent during the period 1970 - 1978 (59). Within these compound feeds protein concentrates are one of the most important and certainly essential factor in the growth and yield of animals concerned.

The animal feed industry in the developed countries of Western Europe, Japan and even of Eastern Europe has come to be dependent on imports of soya, grown almost entirely in a relatively narrow climatic zone in the USA.

The minimum dietary protein requirement in India have been recommended and periodically evaluated by various bodies. These have been critically evaluated by Swaminathan and Parpia (62, 63, 64).

Dietary protein requirement varies from 30 g/day to 92 g/day (at NPU = 50), depending upon age and sex. The national level would depend upon population distibution of different age group, sex, pregnancy etc. In India the requirements for adult male is placed at 55 g/day and national average at 44 g/day.

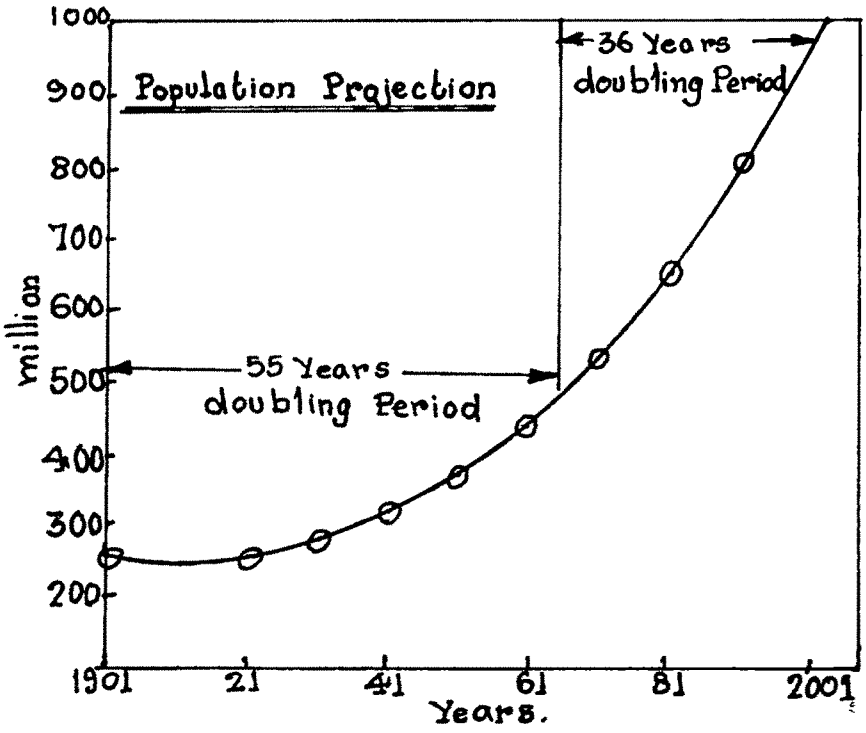
In this country around 350 million people are estimated to live below the minimum levels of food consumption. Per capita production of food grain is around 45.5 gms per day during 84-85 and some of it is lost in handling storage etc. The pattern of consumption shows that average cereal availability including sugar is around 400 gms/day. Obviously the undernorished 300 million people would be consuming much less than the average values.

India is the second most populous country in the world with a density of 239 persons per sq. km., total population being 784 million in 1985. Total agricultural land is about 45 percent (Fig 1). There is very little scope to increase the agricultural land and attempts are therefore being made to increase crop per hectare. This requires additional investments for high yield seeds, fertilizers, pesticides, irrigation facilities etc. Over the period 1960 - 61 to 1980 - 81 rice production increased from 34.6 million tonnes to 53.2 million tonnes and wheat production from 11.0 to 35 million tonnes. Production of cereals as a group increased at annual compound

Fig. 1

INDIA

Population (1985 figure)	785 million
Total No. of cattle	~400 million
Total Land area in million hectares ..	327.614
- Area under forest " " "	70.0 (~21%)
- Net sown Area " " "	172.2
- Towards food gains "	128.12
- Towards Pulses " "	23.55
- Towards oilseeds " "	26.51
- Towards groundnut " "	7.24
- Towards sugarcane " "	3.12



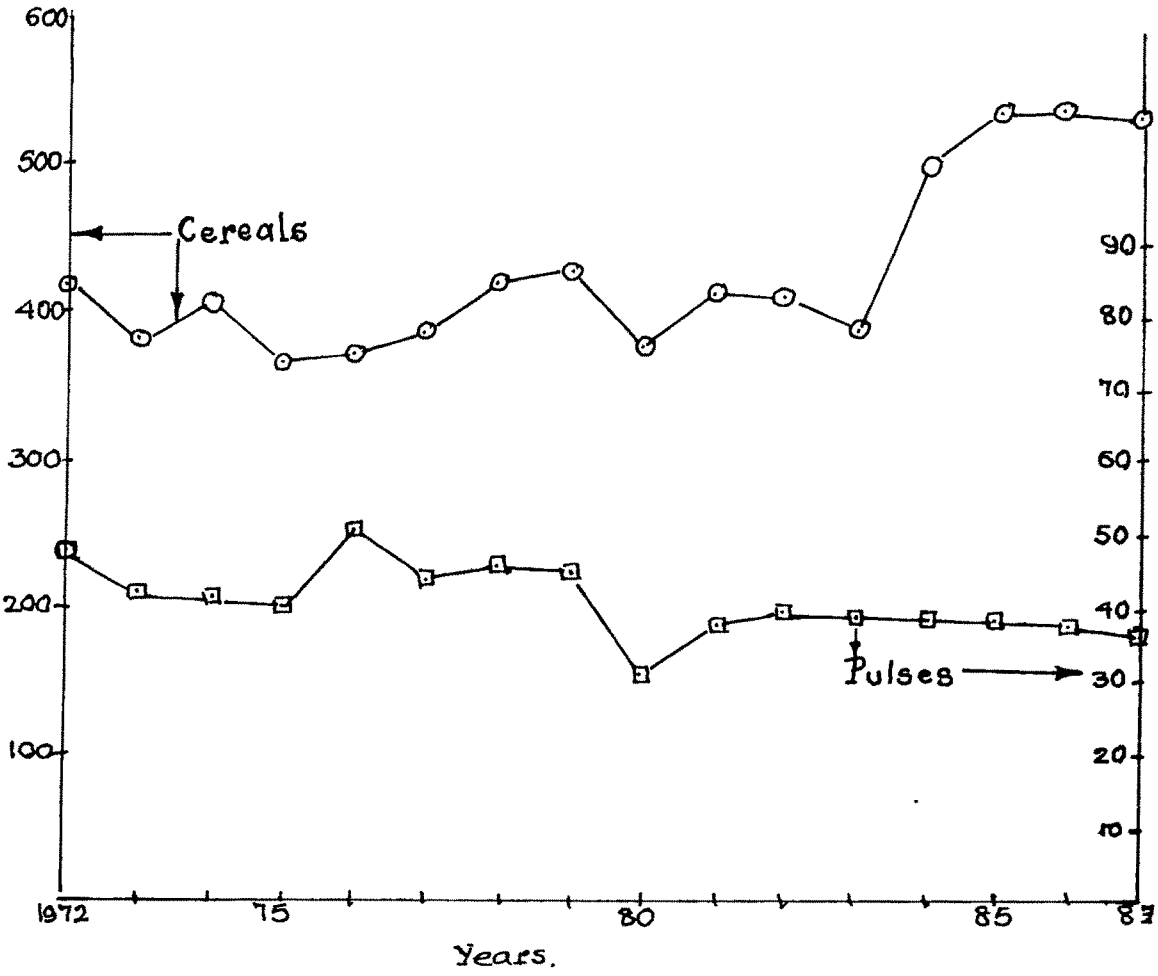
growth rate of 2.94 percent where as that of pulses which is the main source of protein declined at a rate of 0.26 percent and area under pulses cultivation also declined at a compound rate 0.36 percent during the period 60 - 61 to 80 - 81. The per capita availability of pulses declined from 75 gms per day in 1959 to 30.9 gms in 1986 (Fig 2).

Although India ranks second place among pulses growing countries and enjoys first place in area under cultivation, the yield per hectare is quite low.

The deficiency is not confined to human beings alone. The main constraints on the growth of livestick which is an integral part of agriculture in India are, poor genetic consitution of the large majority of our animals and acute shortage of feed and fodder. The total milk production has continued far short of the requirements over the last few decades. Over the period 1960 - 61 to 1980 - 81 milk production increased from 20 million tonnes to 30 million tonnes and during 86 - 87 it is 44 million tonnes. Per capita availability is around 122 g/person/day while

Fig. 2

Percapita Availability of Cereals and Pulses (g./day)

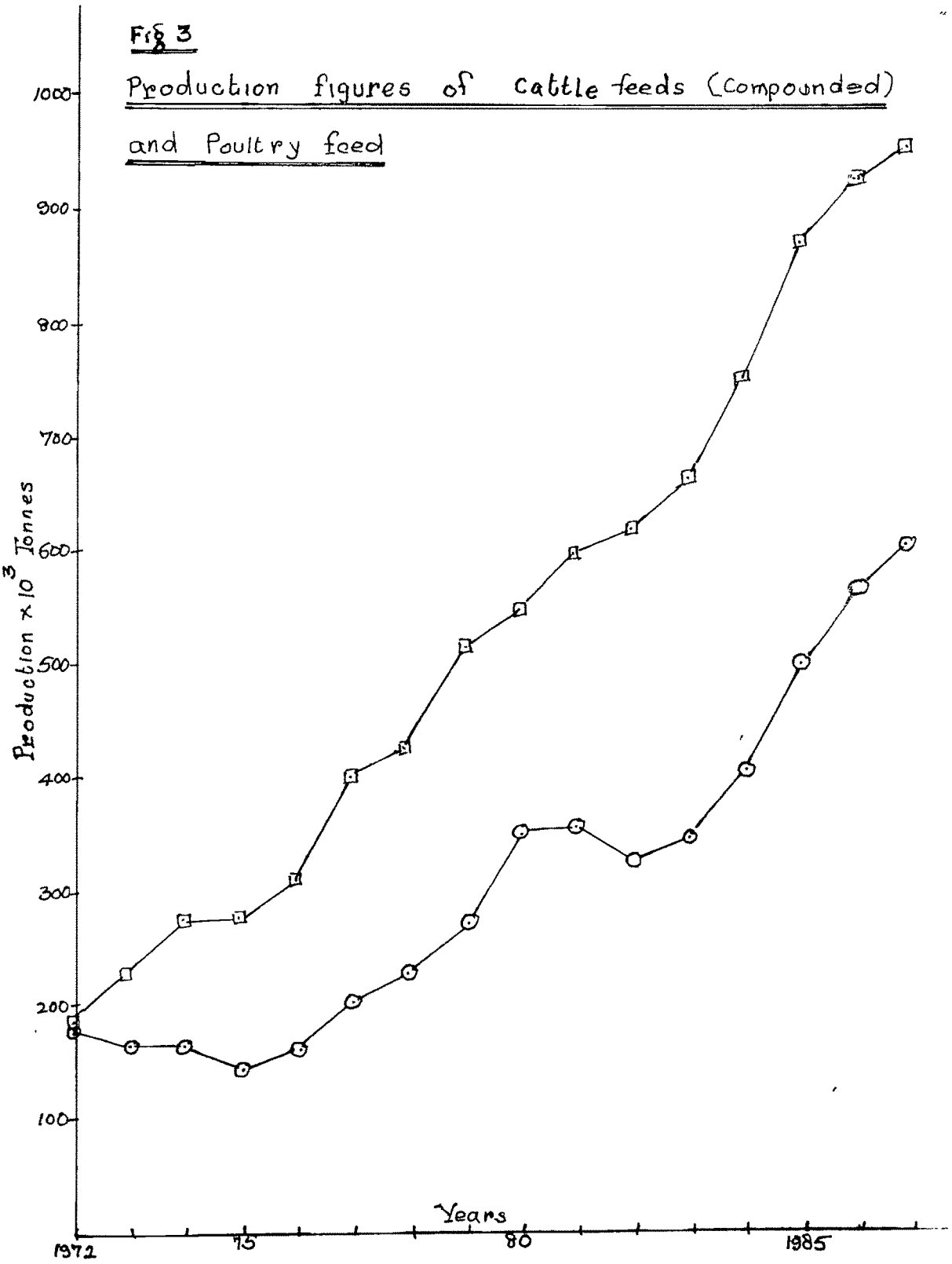


Nutrition Expert Group for Indian Council for Medical Research recommended 300 g/day for pre school children, 250 gms for school children and 200 g for adult man and woman and an additional 150 g for expectant mother. To give this level of consumption the country has to produce at least 100 million tonnes of milk per year. Milk production is essentially dependent upon the fodder production which supplies the basic ration to milching animals. The understanding of nutritional requirements of different animals has led compound animal feed production to rise from 39 x 10^3 tonnes in 1964 to nearly 1500 x 10^3 tonnes in 1986. (Fig 3). Within these compounded feeds protein concentrates are one of the important and certainly essential factor in the growth and yield of animal concerned and groundnut contributes major share.

Although the world over, the oils and fats economy is now dominated by soyabean, sunflower, rape seed and palm oil, groundnut continues to be main stay of India's oil seeds economy. These crops account for about 45% of the total area under oil seeds and almost two thirds of the aggregate production. Given the importance of groundnut

Fig 3

Production figures of cattle feeds (Compounded) and Poultry feed



in the oil seeds economy of the country it is very much desirable that its output shows a steady up trend over the years. Unfortunately it is not so. The highest ever production of 7.28 million tonnes was attained in 83 - 84, there after the output tended to decline.

Oil cake which forms the main supply of protein in the cattle feed is also a potential earner of foreign exchange. We can't afford to lose the earnings of foreign exchange, through this source and at the same time it will be crime to export at the expense of food given to the dumb animals.

In the long run however, a large population has to be served from a relatively narrow resource base and steps have to be taken to increase supply corresponding to population growth. Assuming that the calories demand will be met from the available land, due to the limitations to produce them by any other means, consequently new strategies of protein supply will have to be reckoned with.

I. 2. 6. 2. SINGLE CELL PROTEINS FROM HYDROCARBONS AND ITS RELEVANCE

Single Cell Proteins :

Single Cell Proteins are microbiologically produced protein and has been termed Single Cell Proteins at Massachussetts Institute of Technology (MIT) in 1966 by Prof. Carrol Wilson due to unicellular nature of the microorganism of fungi, yeast and bacterial classes and has clear cut advantages over vegetable and animal proteins.

Two types of microorganisms are actually being tried :

- Bacteria
- Yeasts

These microorganisms are capable of doubling their weight in 2 to 4 hours where as plants like soyabean requires as much as 1 to 2 weeks and animals such as chicken 2 to 4 weeks and cattle 2 to 4 months. Apart from the rate of weight increase the total land requirements would be a small fraction of that needed for agricultural or animal farming. The production of Single Cell Protein is not inhibited by climatic conditions, which limit or

even pose problems in raising of agricultural protein in many parts of the world. Another scoring point in favour of Single Cell Protein is its high nutritive value compared to vegetable proteins due to its protein content with well balanced amino acid pattern comparable to meat protein.

Yeasts are preferred over bacteria especially in hydrocarbon fermentation. The main advantages of yeasts are low pH value of culture which can be used to avoid contamination from unwanted bacteria and the stability of culture for long duration continuous operation. Bacteria has higher protein content, around 70 to 80 % and in the case of yeast it is around 60%. However the high percentage of nucleic acids present in bacteria (around 20%) favours yeast as a good source of protein particularly for human consumption. Besides in the case of bacteria it requires more energy for cell separation due to smaller cell size (around 2 microns when compared to 4-6 microns size of yeast).

Conventional yeast separation methods cannot be

employed in the case of bacterial biomass unless they are floculated. Besides from a psychological point of view yeasts have a potential advantage over Bacteria.

The major problem of microbiological nature are to select suitable microorganisms, which would fulfill the following :

- high yield of cell per unit mass substrate
- high rate of growth (i.e. short doubling time)
- possession of high percentage of protein with all essential amino acids and vitamins suitable for consumption as animal feed and human food supplements.
- ability to grow at as high a temperature as possible.
- should be stable under processing condition. From the biochemical and safety view points the organism should not mutate or become susceptible to infection etc.

I. 2. 6. 2. (b) Survey of Raw Materials :

There are many possible, substrates that can be used

for Single Cell Proteins production. These raw materials fall into three catagories :

- Material that have a high value as a source of energy or are derived from such materials : Methane, Methanol, Ethanol, Diesel fraction, n.paraffinic hydrocarbons.
- Materials that are essentially Waste; Bagasse, Whey sulphite waste liquor.
- Materials that can be derived from plants and hence renewable sources; Starch, Sugar, Molasses, Alcohol and Cellulose.

The main attraction of these microbes for proteins production in the case of hydrocarbon fermentation is that they thrive only on waxy components present in the kerosine and Diesel oil fraction and these waxy components must be extracted to improve the quality of fuels and lubes and as such have low market value.

I. 2. 6. 2. (c) Hydrocarbon fermentation :

Hydrocarbon fermentation could be carried out either by :

- Subjecting straight away the kerosine or diesel oil fraction into the fermentor in which case the n.paraffinic hydrocarbons present in the fraction is assimilated by the yeast cells thereby microbial dewaxing of the fraction takes places inside the fermentor and the oil coming out has better flow properties.
- Feeding pure n.paraffinic hydrocarbons previously separated from the petroleum fraction either by urea adduction or adsorption on 5 A type molecular sieves in which case the microbial cells come in contact with only assimilable hydrocarbon and the chances of yeast cells coming in contact with toxic aromatic hydrocarbons does not arise.

As starter feed a specially cultured inoculum is used. Fermentation is carried out under full aerobic condition so that the biomass production is favoured but not the intermediate products that would arise under oxygen limiting condition.

Unlike in growth of cultures in wholly aqueous media the presence of an immiscible hydrocarbon phase creates

special problems. The two phases in petroleum fermentation are :

- the continuous aqueous medium where nitrogen phosphorous, potassium based nutritive salts, trace elements and vitaminized growth fractions are dissolved.
- the gas oil or other petroleum fraction.

From the chemical engineering point of view this means processing a four phase system i.e. aqueous growth medium, hydrocarbon, microorganisms and air in such a way that the microorganisms are constantly able to satisfy under the best conditions their requirements of a carbon source of nutritive salts dissolved in water and of oxygen.

A second major difficulty is the greater need for oxygen in the case of hydrocarbon fermentation. As hydrocarbon molecule have no oxygen, the oxygen supply delivered to the organisms by bubbling of air through the medium must be higher. It should be atleast three times greater on a hydrocarbon substrate than on a sugar substrate as sugar molecule contains 50% oxygen.

Separation of yeast cells in a pure form, uncontaminated with residual hydrocarbon has also posed many difficulties. This can be achieved by centrifuging as well as by suitable solvent treatment.

These disadvantage are off set, however by an important advantage because all of the oxygen required by the cells is supplied by the air, their consumption of the carbon supplying substrate is correspondingly reduced. The rate of production of yeast is twice as great on hydrocarbon as it is on sugar. Under favourable condition a kilogram of assimilable paraffinic hydrocarbon will produce a kilogram of yeast where as a kilogram of sugar yields only half a kilogram of yeast.

The formation of cellular mass i.e. cell multiplication requires energy and this is supplied by substrate oxidation. A significant proportion of the carbon of the substrate goes to the formation of carbondioxide and the balance for the formation of cell mass. Both of these are highly exothermic reactions (8000 to 10000 k. cal/g. product). Consequently adequate heat removal is a vital process requirement.

Control of the growth environment does not impose the stringents requirements of microbial processes employed for the production of antibiotics and similar products and growth can be brought about under low order aseptic conditions.

There is one great hurdle one has to face before Single Cell Proteins is introduced in the market. In India traditional food habits have deep roots and many socio-scientific problem have to be solved before Single Cell Proteins can become a commonly accepted food. Another problem which has engaged the attention of scientists is the possible toxicity of SCP. In order to overcome these hurdles and to popularise SCP it is necessary to undertake acceptability tests on laboratory animals and large scale field trial experiments on farm animals with locally produced product.

I. 2. 6. 2. (d) Acceptability Tests and its Significance :

World's population explosion has led scientists to the examination of a variety of unusual food sources and Single Cell Protein (SCP) has attracted attention in

recent years. In spite of the widespread interest in this sources of food and in spite of the relatively large investments in development of protein products from Single Cell Organisms, the amount of information available to evaluate the nutritive value of these Single Cell Protein products is remarkably small. Unless nutritional factors in Single Cell Protein are studied, the efforts in developing a process for the production of protein concentrate from petroleum hydrocarbons, go waste.

The principle metabolic area in which Single Cell Protein is useful is in protein metabolism. This, of course, means ability of a food product to supply aminoacids, both in the amount needed and in the form and pattern required for optimal utilization.

The determination of protein in such products is usually performed by analysis for total nitrogen and then multiplication by a factor of 6.25 to convert nitrogen to protein assuming that the average protein contains 16% nitrogen. The results of several studies and reviews indicated that 8 to 12% of the total nitrogen in yeast

cells in due to purines, 4% to pyrimidines, 0.5% of choline, 0.5% to glucosamine, and smaller portions to other non-protein constituents (65; 66; 67). Therefore only about 80% of the total nitrogen of the yeast cells is in the form of proteins. However an accurate knowledge of the detailed composition of the organism in essential for proper evaluation.

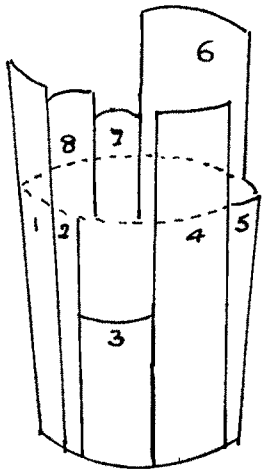
A more accurate evaluation may be obtained by direct analysis for the constituent amino acids of Single Cell Protein.

The estimates of protein needs are given as the amount of body protein that has to be replaced from the diet. However few dietary protein are fully utilized by the body. Proteins are evaluated for use in the diet either by biological or by chemical means (68; 69). The biological tests are in general based on the efficiency of the protein used to replace body protein, when it is fed as the sole dietary source of protein. A protein source fully utilized without wastage is said to have a Biological Value (BV) or Net Protein Utilization (NPU) of 100.

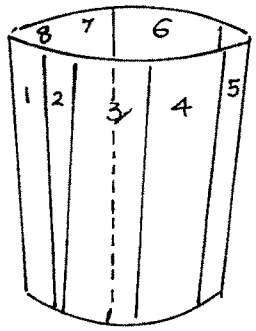
In general, the efficiency of a protein source reflects the proportions of its amino acids. Those proteins with an amino acid composition closely resembling that of the ideal pattern required by the body will be well utilized, whereas those with pattern differing significantly from that of the ideal proportions have a lower efficiency of utilization. The essential amino acid present in lowest concentration relative to needs becomes the factor that limits utilization of all the other amino acids. Thus, in the case of beef protein, phenylalanine is least abundant and reduces the efficiency of the protein in replacing body protein to 69% of an ideal mixture of amino acids. In the case of wheat gluten, a typical plant protein, deficiency of lysine reduces utilization to 33% of that of an ideal mixture of amino acids.

Figure(4 a) describes amino acid pattern of wheat flour and can tell vividly that lysine is the most deficient amino acid as compared with ideal proportion illustrated in (Fig 4 b). Consequently, the rest of the amino acids no matter how much they were contained, cannot

Fig. 4.



WHEAT PROTEIN
(a)



IDEAL PROTEIN
(b)

1. Phenylalanine
2. Tryptophane
3. Lysine
4. Valine
5. Metheonine + Cystine
6. Leucine
7. Threonine
8. Isoleucine.

Essential Amino-acid pattern of Wheat and Ideal Protein

be used by the body, but go waste just like the bucket is unable to hold water more than the level of lysine.

These chemical estimates of utilization are termed chemical scores and agree tolerably well with the results of the biological tests. The chemical score of the most of the Single Cell Protein products is reasonably high and compares well with meat and milk. Some indication of the validity of these analytical evaluation may be obtained in the observation that measured biological values compare reasonably well with the calculated figure. This relationship can be seriously invalidated, however, when the protein is not well used because of poor digestibility or because amino acids have become unavailable. If an intact cell wall did limit the availability of cell components to the animals digestive enzymes then disruption of cell wall would be expected to increase the digestibility of protein.

It is well recognised that amino acid analysis of food stuffs may not be reliable index of the nutritive value of their protein. In order to obtain reliable

information on the nutritive value of proteins it becomes necessary to assess it in experimental animals and human subjects.

The analytical information which should be available in the initial phase partly concerns the nutritive quality and partly the safety. The proximate composition, amino acid pattern and levels of certain minerals such as calcium, phosphorous etc. provide the data essential for designing balanced diets to be used. The ever increasing amount of literature on toxic substances occuring naturally in foods has contributed a great fear of possible health risks in all types of food and feeds. It has been reported that even such reputable health food like egg, milk, beans, potatoes, tomatoes, groundnut and wheat are known to contain injurious components. Perhaps it may be proved that all what we take above certain limits are injurious to health in one way or the other.

Novel protein sources are often derived from micro-organisms. During the production the materials are often submitted to treatments by heat, solvents or alkali each

of which may impart undesirable or even toxic properties to the final product.

It is essential therefore to demonstrate the safety of Single Cell Protein not only in view of the public health aspects but also in the interest of the producer and the image of this new source of food. Several National authorities and other bodies have drawn up elaborate guidelines and protocols for the various types of information required for the evaluation of the toxicity of food additive and contaminants.

From the various categories of information needed according to the guidelines proposed by protein Advisory Group (PAG) of United Nations, those on chemical composition, protein quality, microbiological status and biological safety form the first part of the programme, and these are usually carried out on laboratory animals. This is followed by controlled feeding studies carried out on certain farm animals.

Determination of protein quality is conducted with a variety of biological, chemical or microbiological methods.

The most relevant information is provided by each one of three biological methods which are widely used by nutritionists. The bio-assays are performed with young rats by feeding the product to be examined as the sole source of protein in the diet at a level providing 10% crude protein. The protein Efficiency Ratio (PER) the Net Protein Utilization (NPU) and the N-balance method and then safety evaluation in laboratory animals, which include sub-acute tests, sub-chronic (90 days) study, chronic (2 years) feeding study, multi generation study, teratogenicity and mutagenicity studies are some of the main tests that are to be covered initially before feeding trial experiments for animal or human consumption is taken up. During the early 1960 a number of babies with no limbs or with soft limb buds (without bones) were born in Europe to mothers who were quite healthy and normal. This was attributed to the consumption of a sleeping pill phalidomide by the mother during the pregnancy. It was non addicting, non toxic and completely safe for the mother but produced unforeseen effects in the baby.

Some researchers feel that excess of certain nutrients can also be harmful to the embryo. The classical example is that of vitamin A, which, in excess amount can cause foetal malformation. Similarly excess of protein in the diet has also been shown to be harmful to the foetus. Those compounds which could produce structural or functional malformation in an embryo in the uterus are called teratogens.

The hazard to man from carcinogens in food can be rated higher than that from teratogens.

Some of the aromatic hydrocarbons present in petroleum fraction are known for their carcinogenicity and naturally fear exists that any food material prepared out of petroleum fraction may be connected with cancer in some way or the other. Perhaps due to these reasons consumer organization in Japan objected to accept the product and Single Cell Proteins from petroleum fraction was banned and two companies in Japan had to suspend their commercial project for nearly three years. With the announcement by the Food Sanitation Council and Advisory Body to the

Welfare Ministry at its meeting in December 1973, that Single Cell Protein from n- alkanes is not harmful to animals, the ban was lifted and further work started. Thus the safety of Single Cell Protein has to be basically demonstrated on the following aspects :

- it is not carcinogen
- it does not affect reproduction rate
- the absence of teratogenic effect (or deformation producing effect on off-springs).
- the absence of mutagenic effect i.e. the fertility is not impaired.

Satisfactory completion of these studies may not provide conclusive proof of safety for long continued use because of species variation, the limited number of animals employed and the objective nature of the test criteria. There is a need for careful observation of the responses in animals on large scale field trial experiments. It is clear that the direct use of Single Cell Protein for human food can only occur in the long term. Much of the current interest however is to indirectly use Single Cell Protein

to obtain its protein value in animals food. There are certain advantages in feeding Single Cell Protein to animals rather than directly to man :

- there is no need to isolate protein and the whole of Single Cell Protein can constitute edible feed.
- Single Cell Protein can be a source of calories as well as vitamins in the animal diet.
- Single Cell Protein in the powder form can be directly incorporated in animal feed constituents where as in the case of human consumption it has to be in the textured form or some other suitable forms.
- nutritional acceptance of Single Cell Protein can be directly demonstrated using experimental animals.

In order to provide information and suggest guidelines for test procedures the PAG issued over the course of time, a series of statements and guidelines (70; 71).

In addition the International Union of Pure and Applied Chemicals (IUPAC) proposed guidelines for testing Single Cell Protein for animal feed (72).

I. 2. 6. 3. THEORETICAL ASPECTS OF MICROBIAL GROWTH

Growth Rate Study

Assuming doubling of the initial biomass X_o in the Volumetric unit of the medium during cultivation in time g, it can be written :

in time 0 the concentration of microorganisms is X_o

in time 1g ,, ,, is $2X_o$

in time 2g ,, ,, is 2^2X_o

in time 3g ,, ,, is 2^3X_o

in time ng ,, ,, is 2^nX_o

hence the final concentration of microorganisms X

$$X = X_o\, 2^n \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

n expresses the number of cell divisions in time t.

Equation $\frac{t}{n} = g$ ..... (2)

therefore expresses the average generation time or doubling time usually determined by doubling of the microbial mass in the culture.

By substituting for n, eqation (1) becomes

$$X = X_o\ 2^{t/g} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

By converting into logarithmic form and rearranging

$$g = \log_e 2\ t/\ \log_e X - \log_e X_o \quad \ldots\ldots\ldots\ldots\ldots \quad (4)$$

equation (3) can be written as

$$\log_e X = t.\ \log_e 2/g + \log_e X_o \quad \ldots\ldots\ldots\ldots\ldots \quad (5)$$

$\log_e 2/g$ is constant during the logarithmic phase and μ may be substituted for this

$$\log_e X = \mu\ t + \log_e X_o \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

This equation expresses the increase of biomass within a certain time and is of the form $y = mx + c$.

On plotting the values of t on the x - axis and of $\log_e X$ on the ordinate a straight line is obtained and the constant μ is the slope of this straight line.

It determines the growth rate of the biomass which is dependent on time and is therefore called specific growth rate.

Its value can be determined either graphically or by calculation using the equation (6).

The specific growth rate or exponential growth rate u is specific for every organism and medium. It is primarily affected by internal factors i.e. the growth capacity of the microorganisms and also on the environment of the microorganisms.

The dependence of specific growth rate on the substrate concentration is expressed by Monod.

$$\mu = \mu_{max} \quad \frac{S}{Ks + S} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (7)$$

This represents an analogy to the Michaelis ± Menten equation for enzymatic reactions. S is the limiting substrate concentration and Ks is the saturation constant, which equals the substrate concentration at which the specific growth rate corresponds to half of the maximum growth rate μ_{max}.

For exact determination of the value of Ks and u_{max} the equation (7) can be written as

$$\frac{1}{\mu} = \frac{Ks + S}{\mu_{max} S} = \frac{1}{S} \cdot \frac{Ks}{\mu_{max}} + \frac{1}{\mu_{max}} \quad \ldots\ldots\ldots\ldots\ldots \quad (8)$$

When $\frac{1}{\mu}$ is plotted as the ordinate and $\frac{1}{S}$ as the

abscissa the straight line obtained defines the value of $\frac{1}{\mu_{max}}$ on the ordinate and $\frac{1}{Ks}$ on the abscissa.

The limiting factor for the growth rate is not only the substrate concentration but also on the density of the population.

Continuous cultivation :

In a closed system with constant volume of the medium the concentration of the substrate in substrate limiting growth constantly changes and gradually decreases. If it decreases to such an extent that the saturation constant already gains importance in the fraction $\frac{S}{Ks + S}$, the cultivation passes from the logarithmic phase into the negative acceleration of the growth rate until growth stops at practical exhaustion of the substrate. By supplying fresh substrate and withdrawing the products of metabolism it would be possible to maintain the culture permanently in the state of maximum multiplication.

Continuous cultivation presumes continuous flow of the medium, i.e., continuous inflow and outflow. The semi

continuous process is characterized by the regular withdrawal of part of the medium at constant time intervals.

The kinetic equations expressing the condition of multiplication in the batch process is the basis for the elucidation of the mathematical relation determining the conditions governing homogeneous semi-continuous and fully continuous process for the production of microbial mass. It has to be assumed that the content of the fermentor is perfectly agitated, the culture is homogeneous and its properties are also practically constant at high concentration of the substrate limiting growth. The other substances influencing growth are also present in excess. The specific growth rate μ can therefore be presumed to approach μ_{max}.

The equation (6) can be write in the form :

$$X = X_o \, e^{\mu t} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (9)$$

The equation in this form is known as the equation for natural growth. By its derivation by time the relation determines the instantaneous increment of microorganisms during cultivation.

$$\frac{dx}{dt} = \mu X \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (10)$$

It is assumed that the specific growth rate is maintained on a constant level in the vicinity of the maximum growth rate by a sufficiently high concentration of the substrate limiting growth. This can be attained with the semi continuous cultivation method by regular withdrawal of part of the spent medium in certain time intervals and its replacement by fresh substrate without microorganisms. The withdrawal part is designated V. At sufficiently high substrate concentration immediately before withdrawal, so that effect of Ks is neglible, the increase of growth rate on addition of fresh substrate can also be neglected and μ can be assumed constant and very close to μ_{max} for the total cultivation time.

As long as the amount of microorganisms together with the substrate concentration varies within the range of the logarithmic growth phase during the single period, the specific growth rate is constant. The number of periods in the unit of time can be different and where it is sufficiently high, semi continuous process passes into a fully continuous process.

To comprise the conditions governing such a process the total microbial mass in the fermentor immediately after withdrawal of the spent medium can be assumed to be defined by:

$$X = X_o e^{\mu t} \quad \text{_____} \quad V. X_o e^{\mu t} \quad \text{____} \quad (11)$$

total amount = growth from initial amount — withdrawal

Provided time t = 1 hour and the withdrawal volume V reffered to this time and called flow rate F, then

$$X = X_o e^{\mu} (1-F) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (12)$$

Where the flow rate F is the amount of medium withdrawn per hour from the total fermentor volume and possesses the dimension $L\ h^{-1}$.

Another important term is the dilution rate D, i.e., the rate of exchange of the medium in the fermentor. It is given by the ratio of flow rate to the total medium volume in the fermentor.

$$D = \frac{F}{V}\ h^{-1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (13)$$

If the value of the fermentor V = 1 then the flow rate per hour simultaneously indicates the dilution rates i.e. D = F then

$$X = X_o e^{\mu} (1 - D) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (14)$$

Assuming the instantaneous increment μX is immediately compensated by the outflow of biomass with medium DX the condition of the steady state is necessarily expressed by

$$\frac{dx}{dt} = \mu X - DX = 0 \quad \ldots\ldots\ldots\ldots \quad (15)$$

increase= growth - output

and hence μ - $D = 0$ i.e. $\mu = D$

i.e. the specific growth rate is equal to the dilution rate. This is always respected in single stage continuous cultivation.

Self regulating capacity of the continuous process :

The cultivation process has been considered only under the conditions of the steady state, the dilution rate being in equilibrium with the specific growth rate.

$D = \mu$ (for the continuous process).

When the dilution rate is increased above the specific growth rate.

i.e. $D \gg \mu$ then $\frac{dx}{dt} = \mu x - Dx < 0$

The value of $\frac{dx}{dt}$ is negative, i.e., instead of steady state being established the biomass decreases in the system. The microorganisms are being washed out and their concentration in the medium decreases. The substrate is not utilized and its concentration increases. The increased substrate concentration positively influences the specific growth rate and its value increases according to the equation $\mu = \mu_{max} \frac{S}{Ks + S}$. Upto its limit, where the specific growth rate equals the maximum growth rate. Until the value of the dilution rate surpasses the value of the maximum specific growth rate a new equillibrium between the dilution rate and specific growth rate is established in the system.

When the dilution rate D surpasses the value of the maximum specific growth rate all microorganisms are washed out from the fermentor in a certain time.

At decreasing dilution rate on the contrary the retention time of the medium in the tank is prolonged, The microorganisms are given more time to utilize the substrate, its level therefore decreases and the concentration of microorganisms increases in proportion. The lower level of the substrate influences the growth rate and the specific growth rate therefore decreases. A new steady state is established in the system. The continuous process obviously possesses a self regulating capacity in the range upto the maximum specific growth rate.

Yield is defined as the ratio of the microbial mass formed and the decrease of the substrate.

$$Y = -\frac{\Delta X}{\Delta S} = \frac{dx}{ds} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (16)$$

By substituting into this relation from the combined equations :

$$\mu = \mu_{max} \frac{S}{Ks + S} \quad \text{and} \quad \frac{dx}{dt} = \mu X$$

it becomes

$$-\frac{ds}{dt} = \mu_{max} \frac{X}{Y} \cdot \frac{S}{Ks + S} \quad \ldots\ldots\ldots\ldots\ldots\ldots (17)$$

expressing the relation between substrate consumption and biomass production. As already mentioned the condition of the steady state of the microbial material balance is expressed as

$$\frac{dx}{dt} = \mu x - Dx = 0$$

increase = growth - output

$$\frac{dx}{dt} = \mu_{max} \cdot X \cdot \frac{S}{Ks + S} - DX = 0 \quad \ldots\ldots\ldots (18)$$

The relation expressing the substrate balance for the steady state can be established.

$$\frac{ds}{dt} = DS_o - D S - \mu_{max} \frac{X}{Y} \cdot \left(\frac{S}{Ks + S}\right) = 0 \quad \ldots (19)$$

increase of substrate = input - output - consumption of substrate

Where S_o is the substrate concentration in the inflowing medium and S the actual concentration of the substrate in the tank and therefore also in the outflowing medium.

For substrate consumption the value of the equation (17) is substituted. Since equations 18 and 19 equals 0,

the values of X and S can be calculated for the condition of steady state. For the calculation of microorganisms X one can start from the equation (19).

$$D\ (S_o - S) = \frac{X}{Y} \cdot \mu_{max} \cdot \left(\frac{S}{Ks + S}\right) \quad \ldots\ldots\ldots\ldots (20)$$

Since in the steady state $D = \mu_{max} \left(\frac{S}{Ks + S}\right)$ as follows from equation(15) and (7)

$$X \left(\mu_{max} \frac{S}{Ks + S} - D\right) = 0 \quad \text{and}$$

$$D = \mu_{max} \frac{S}{Ks + S}$$

from which one can calculate the substrate concentration

$$S = Ks \frac{D}{\mu_{max} - D} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (21)$$

and

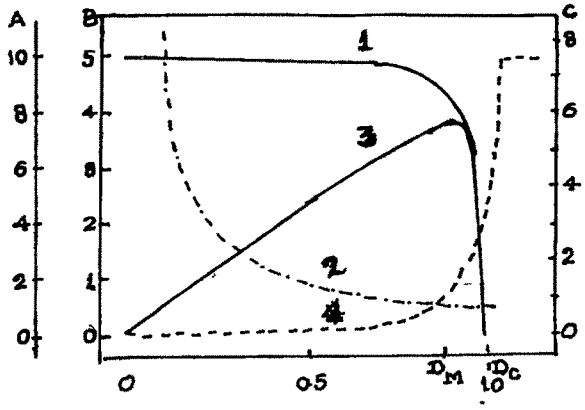
$$X = Y (S_o - S) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (22)$$

The values of Ks and μ_{max} are determined experimentally in a batch process and so also the yield Y. If the values are known the self regulating capacity of the continuous process can be plotted. This evaluation was

carried out by Hubert and is given in the diagram (Fig 5) for tentatively selected values of the yield (Y), the maximum specific growth rate (μ_{max}) and the saturation constants (Ks). From these data the relation between substrate concentration, specific growth rate, the amount of microorganisms in the fermentor and in the outflow were obtained in dependence on the dilution rate.

It can be seen at increasing dilution rate the generation time as well as the concentration of microorganisms in the fermentor decreases until total washing out. This takes place when the value of the dilution rate exceeds the value of the maximum specific growth rate (μ_{max}). The concentration of the substrate simultaneously increases until equalization to the original concentration of the substrate S_o flowing into the fermentor.

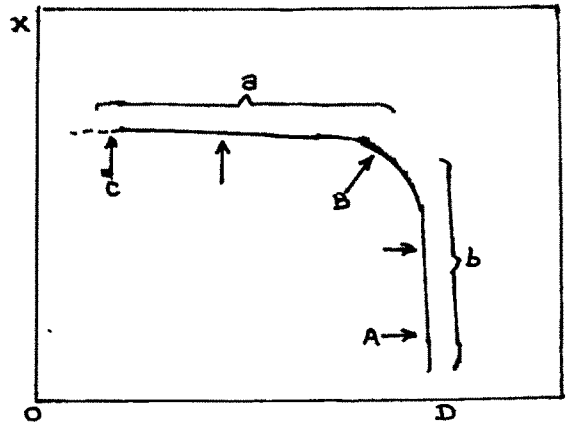
The diagram shows in which internal the self regulation capacity of the system can be taken into consideration and by what it is limited. As long as the dilution rate at the corresponding specific growth rate is smaller than the value of the growth constant (μ_{max}) a steady state



Theoretical relations determining the steady state.

A- Substrate concentration (S) g/L
B- Concentration of microorganisms (X) g/L.
C- Doubling time (g)
D- Dilution rate

Curve 1- Variation of biomass concentration (X)
" 2- Variation of doubling time (g)
" 3- Variation of production of microorganisms (DX)
" 4- Variation of substrate concentration (S)



Ideal concentration of microorganisms in the steady state in dependence on the dilution rate.

Part B-C (a) range suitable for Chemostat
Part A-B (b) range suitable for Turbidostat.

Fig. 5

with an exactly defined concentration corresponds to every change of the substrate concentration and vice versa. Continuous cultivation of microorganisms for experimental work can be controlled in two ways:

1. by maintaining the concentration of the growth limiting substrate in certain level.
2. or by keeping a constant concentration of cells in the medium.

As long as the value of the dilution rate is noticeably lower than the maximum growth rate, the steady state of the limitation can be maintained with the aid of the substrate concentration even at the conceivably small oscillation of the parameters u, X and Y, given by the properties of the biological material and of the dilution rate, caused by experimenterror. Only a small change of the concentration of the substrate and of the microorganisms corresponds to even considerable changes of the dilution rate.

Productivity :

Curve 4 in the Figure (5) represents the changes of the amount of microorganisms leaving the fermentor. This amount is given by the product of the dilution rate and the concentration of microorganisms on the outflowing liquid and therefore equals the value of DX. The value DX actually expresses the productivity of the system, i.e. the amount of microorganisms produced in the volumetric unit in the unit of time.

Since the volume of productivity depends on the dilution rate as well as on the concentration of microorganisms in the fermentor the productivity of the system grows with the increasing dilution rate (D), upto the point DX_m, i.e., the point of maximum productivity. It is evident from the productivity curve that maximum productivity is attained in the range close to the value of the maximum dilution rate.

The point maximum productivity does not concide with the maximum yield referred to the inflowing substrate in Single stage continuous cultivation. The growth of a

certain amount of microorganisms at maximum productivity therefore requires a greater amount of substrate (S_o) than at lower productivities (i.e. at lower dilution rate). Maximum productivity is therefore always connected with an increased concentration of the substrate in the outflowing medium. For this reason it is always necessary to differentiate between the maximum productivity DX_m and the optimum productivity. The optimum productivity must be determined for every particular case. It depends on the price of the raw materials, on the possibility of further use of the substrate contained in the outflowing medium as well as on the process cost including investment cost.

I.2.6.4. MECHANISM OF UPTAKE OF N.ALKANES BY MICROORGANISM

The mechanism of liquid hydrocarbon uptake by microorganisms represents an important problem. The kinetic model for cell growth on hydrocarbon should be based on the mechanism of hydrocarbon uptake.

In microbial culture with liquid hydrocarbon of low solubility in water, there exist two liquid phases, i.e. the aqueous and the hydrocarbon phase.

The microbial assimilation of hydrocarbon and the growth on it are considered to be strongly associated with the microbial affinity for hydrocarbon.

The first step of alkane assimilation by microorganisms is the exogeneous alkanes by the cells and the further transport to the site where the alkanes undergo the initial oxidation. The mechanism of alkane transport has been extensively studied (73; 74).

As n.alkanes are almost insoluble in water, the means used to effect substrate transfer onto and into the cell

is of fundemental interest and is mainly supported by direct contact of droplets or aggregates of hydrocarbons with the microorganisms.

" Accommodated " molecule, " Pseudosolubilization " and " Colloidal emulsion " with submicroscopic droplets are some of the terms that have been used in the description of this phenomenon. The hydrocarbons are solubilized as submicroscopic " micelles ".

Regarding the mechanism by which an emulsion of water isoluble substrates is produced, a distinction is made between a macro-emulsion which is produced by the shear stress of mechanical agitation and a micro emulsion which is considered to be build up of an additional substance produced by the microorganisms. The macro emulsion contains droplets with a size of 1-100 µm. The micro emulsion consists of highly dispersed droplets with diameters of 0.01 - 0.5 µm and is build up at the liquid - liquid interface in the presence of emulsifiers, which might eventually be formed spontaneously by the microorganisms.

On the other hand, micelles are aggregates of molecules of surface active agent, which are saturated with solubilized hydrocarbons.

The mechanism of quasi - spontaneous emulsification was used as an explanation by several research groups. It has been established that the structured mechanical barrier is responsible for the stability of the emulsion towards coalescence. This barrier is constituted by microemulsions formed spontaneously at the oil - water interface in the presence of surface active substances. The mass transfer of some emulsifiers through the interface and the decrease in local interfacial tension lead to quasi - spontaneous emulsification and the formation of highly dispersed microdroplets at the interface.

Analagous films of microemulsion which are formed at the interface of microemulsions may be taken out of this interface mechanically.

The ability to produce emulsifying surfactant compounds at the cell membrance is one of the main determining characteristics of hydrocarbon emulsification.

The microscopical observations of Mimura et al (75) and Blanch and Einsele (76) showed that Candida petrophilum and Candida tropicalis adhered to the oil phase in cultures with hydrocarbons as a substrate and that the cells and oil droplets formed agglomerates, so called flocs. Einsele, Schneider and Fiechter (73) in studies with electron microscope observed the submicroscopical oil droplets adhering to the cell wall. The affinity of the cell surface to hydrocarbon was investigated by Kaeppeli and Fiechter (77) using the yeast strain Candida tropicalis ATCC 32113.

The cell grown on glucose showed a 25% lower adsorption capacity compared to those grown on n.alkanes and were more sensitive to the quality of the emulsion. There was a widening of the gap of the adsorption capacity with decreasing quality of the emulsion for cells grown on glucose and on hydrocarbons. These results suggested to Kaeppeli and Fiechter (77) that there are changes in the cell surface related to the growth substrate and that this may partly explain the formation of flocs during the

shift from glucose to hydrocarbons as reported by Hug, Blanch and Fiechter (78). The properties of the cell surface of C. tropicalis ATCC 32113 were investigated by Kaeppeli (77). It was shown that there were two peculiarities important for the interaction between the cell surface and the hydrocarbon :

1. Porous structure of the surface layer and
2. Formation of a mannan - fatty acid - complex during growth on hydrocarbons thereby increasing the lipophility of the surface.

The porous structure of the cell surface is considered not to be affected by the substrate charge. An emulsifying agent which was homologous to the mannan - fattyacid - complex at the cell surface was isolated from the medium.

Osumi et al (79) investigated the ultrastructure of the cell surface of Candida tropicalis and that of C. albicans growing on n.alkanes using field emission scanning electron microscope. A curious protrusion of 100 - 200 um in diameter was observed on the surface of the yeast cells. The protrusions, consisting of small subunits

of about 50 µm in diameter were scattered at a regular distance from each other on the cell surface. The sectioned view of the cells grown on n.alkanes obtained by transmission electron microcopy showed the existence of slime - like outgrowths on the periphery of the cell wall. These outgrowths which were observed as electron dense layers, developed across the cell wall thereby producing channels that connected to the cell membrane. The slime - like outgrowth was suppose to correspond to the above mentioned protrusion on the cell surface.

Meissel et al (80, 81) have observed electron microscopically special channels in the cell wall of alkane grown cells of C. tropicalis. Similar channels accompanied by slime - like outgrowths have also been detected by Fukui and Tanaka (58) together with protrusion on the cell surface of alkane grown C. tropicalis at an early phase of growth.

The protrusions or outgrowths do not seem to be alkanes or lipids which are simply attached to cell wall, because these constituents cannot be removed by washing the cells with organic solvents or detergents.

The ability to produce emulsifying surfactant compounds at the cell membrane is one of the main determining characteristics of hydrocarbon assimilation. It has been established that lipopolysaccharide - isolated from the walls of the hydrocarbon utilizing microorganisms - play an important part in the process of hydrocarbon uptake (82).

Using Candida intermedia, Candida tropicalis and Saccharomyees cerevisiae, Miura et al (83) investigated the affinities of the hydrocarbon utilizable and unutilizable microorganisms for liquid hydrocarbon by measuring the degree of adhension between the cell and hydrocarbon. Alanine - precultured cells were used in the investigation of their adherent characteristics to hydrocarbon, since they are considered not to contain the hydrocarbon before the investigation. The results indicate that C. intermedia and C. tropicalis, which can utilize the hydrocarbon, adhere well to the hydrocarbon but that S. cerevisiae which cannot utilize the hydrocarbon is not adherent. The number of adsorbed cells per unit surface area of hydrocarbon was not appreciably different for C. tropicalis

and C. intermedia whereas clumps were formed by C. intermedia and hydrocarbon, C. tropicalis did not form clumps with hydrocarbon.

Miura et al (84) conducted experiments to determine adhesive force between cells and hydrocarbon using two different strains Candida intermedia and Candida tropicalis and they came to the conclusion that the affinities of the hydrocarbon - utilizable microorganisms for hydrocarbon are different.

Pathways for hydrocarbon uptake :

In hydrocarbon fermentation, the three different possible pathways considered for hydrocarbon uptake are as follows :

1) uptake of dissolved hydrocarbon in aqueous phase.

2) direct contact of cell with submicron oil droplets i.e. the accommodated oil droplets proposed by Aiba et al (85) and

3) direct contact of cells with large oil drops.

It was reported by Erdstieck and Rietema (86) that the uptake of dissolved hydrocarbons should not be neglected for the microbial growth when undecane and dodecane are used as a carbon substrate.

Yoshida Yamane and Yagi (87, 88) also showed that the utilization of dissolved hydrocarbon might contribute to the microbial growth when decane and shorter chain hydrocarbon are used as a carbon source.

Chakravarthy et al (89) observed that the microbial cells did not stick to the hydrocarbon particles during growth on emulsified solid paraffins. They assumed that the growth occured on dissolved substrate and that the dissolution of the substrate was helped by a metabolite product by the cells.

On the other hand Johnson (90) denied the possibility of microbial uptake of hydrocarbons dissolved in the aqueous phase, inview of the very low solubilities in water of n-paraffins heavier than C_{10} and proposed that in fact, the microorganisms took up liquid hydrocarbons by direct contact with hydrocarbon drops. Aiba and Haung (91)

based on their experiments carried with a strain of Candida guillermondii proposed that the uptake of dissolved hydrocarbon can be neglected when comparatively longer chain hydrocarbons such as hexadecane are used as a substrate.

Several researchers (92, 93, 94, 95) reported that the yeast cells grew mainly at the interface between oil and water and that the growth was limited by the interfacial area.

Katinger (95) reported that the interfacial area per unit oil fraction increased as cultivation proceeded. Wang and Ochoa (94) and Prokop et al (93) showed that the cells caused an increase of the interfacial area.

It has been suggested by Aiba et al (85, 91), Moo Young, Shimiza and Whitworth (96, 97), Goma et al (98), Yoshida et al (99) and Hisatsuka et al (100) that the liquid hydrocarbon available to the cell were mainly submicron droplets. The microorganisms used by these researchers seem to have low affinities for hydrocarbons.

Einsele et al (101) reported that there might be free cells, cell which might adsorb submicron droplets, and cell attached into large oil drops in the hydrocarbon fermentation. Bukhuis and Bos (102) investigated the relation between the growth rate of Candida tropicalis and the size of the oil droplets and showed that the growth rate was the lowest for the oil droplets size of 20 to 25 μ and that it was about twenty times higher than this minimum for the droplets size of 16 μ but only about five times higher at the droplets size of 29 μ.

Nakahara et al (103) showed through microscopic observation and the results of batch cultivation in the tower system that cells attached to large oil drops formed flocs as has been reported by Mimura et al (75) and Einsele et al (101). However it was observed that a significant fraction of the cell were free from large oil drops. About 30% of the cell in the culture broth precipitated during centrifugation. This suggest that more than one half of the cell may be attached to large oil drops. Therefore both processes i.e. direct contact

of cells with large drops and utilization of submicron droplets may be important for hydrocarbon uptake by microorganisms.

Nakahara et al (103) also showed that the interfacial tension between oil and water decreased sharply as cultivation proceeds and as the size of the oil drop decreases. They investigated the effect of various fatty acids as well as cell density on the interfacial tension, demonstrating that long chain but that palmitic acid has only a slight effect on it. They suggested that in batch fermentation the relative contribution to the microbial growth of large drops and submicron droplets may change as the cultivation proceeds because of the decrease in drop size and due to an increase of pseudo solubility (103) as the result of substantial change of the interfacial tension. Shah et al (104) came to the same conclusion.

Miura et al (84) investigated the pathway for liquid hydrocarbon uptake using several microorganisms with different affinity for hydrocarbon and showed that the

pathway for liquid hydrocarbon uptake depends upon the affinity of the microorganisms for hydrocarbon. From the different results obtained it is concluded that the quantity of dissolved hydrocarbon utilized is negligible compared with the quantity of drop and accommodation form hydrocarbon utilized when comparatively longer chain hydrocarbon such as decane are used as substrate. In this work drop form hydrocarbon refers to hydrocarbon drops of longer size than the cell and the accommodation form hydrocarbon refers to hydrocarbon droplets of smaller size than the cell.

From the different results obtained it is considered that microorganisms with high affinity for hydrocarbon can utilize the drop form hydrocarbon almost equally as well as the accommodation forms, while microorganisms with low affinity utilize the accommodation form more effectively.

Lebeault et al (105) reported a dehydrogenation of n-decane in mitochondrial extracts, however Van der Linden and Hugdregtse (106)as well as Liu and Johnson (107)

have found alkane oxidizing enzymes localized in the cytoplasmic membrane.

Ludwik et al (108) observed the following ultrastructural features of a strain of Candida lipolytica on electron micrographs :

1) The surface of the yeast cell wall after growth on hydrocarbon is covered with a thin layer of hydrocarbons which penetrate through the cell wall to the cell membrane. The accumulation of hydrocarbon is especially marked in yeast cells grown on gas oil. Hydrocarbon accumulated on the surface of the cytoplasmic membrane.

2) The cytoplasmic membrane of cells grown on hydrocarbons is always thicker and clearly visible and contains deep invaginations and digital projections which represents an increase of the surface of the cytoplasmic membrane. Pinocytotic Vesicles were frequently observed at the ends of deep invaginations, suggesting the possibility of an active translocation of hydrocarbons into the cytoplasm.

3) Yeast cells grown on hydrocarbons contain more abundant endoplasmic veticulum.

4) Cells grown on media with hydrocarbons contain more fat vacuoles than do cells grown on glucose containing medium.

5) Yeast cells grown on hydrocarbons have more mitochondria which frequently contain an intra mitochondrial vacuole.

6) The cell wall of these yeasts is thinner than those cells grown on glucose.

7) The cytoplasm of cells grown on hydrocarbons is more electron dense and contains more ribosomes.

8) Cells on glucose contain numerous glycogen granules, where as the hydrocarbon grown cells contain less polysaccharides and more fat vacuoles.

From these observations the authors conclude that hydrocarbons penetrate the cell wall of C. lipolytica and are concentrated at the surgace of the cytoplasmic membrane bringing about numerous morphological changes of

the cell and that further more the cytoplasmic membrane seems to play an important role in the metabolism of hydrocarbon as well as in their transport into the cell.

In addition Munk, Dostalek and Volfova (109) demonstrated on electron micrographs, the penetration of hydrocarbons into the yeast cell and estimated the velocity as well as reversibility of this process by using tritium traced hexadecane.

Kennedy and Finnerty (110) have observed an intra cytoplasmic accumulation of hydrocarbon in Micrococcus cerificans, indicating that the alkane - oxidizing enzyme was located with in the cell.

Vol Fova et al (111) showed that protoplasts of C. lypolytica were unable to assimilate hydrocarbons. However Lebeault et al (112) demonstrated that protoplasts of C. tropicalis oxidized decane and tetra decane. It appears that the location of the enzyme responsible for hydrocarbon assimilation is different depending on the kind of microorganisms involved.

There are many reports of increased lipid content of both bacteria and yeast on hydrocarbon (78, 90, 113, 114, 115, 116).

The role of cellular lipids in hydrocarbon assimilation has been discussed by several researchers. Dunlap and Perry (114) proposed that on the basis of solubility of hydrocarbons the cellular lipids play an important role in hydrocarbons assimilation, intermediates of alkane degradation provided a solvent for the insoluble alkanes. Vestal and Perry (117) also suggested that an increased lipid content was necessary for the uptake of and the accumulation of liophillic substrates. The functional role of cellular lipids in hydrocarbons, was discussed by Hug et al (78). When grown on hydrocarbons, the yeast Candida tropicalis contained twice as much lipid as when grown on glucose.

In the transient continuous culture phase following a substrate change from glucose to hexadecane, an adaptation occured. The lipid concentration per cell increased greatly during that transient phase. The cause of that

adaptation was assumed to be due to both induction of the enzymes required for hexadecane oxidation and the necessity of transporting this substrate to the site of enzymatic action. These authors (78) proposed that the role of lipid in the hydrocarbon assimilation process is to provide a hydrophobic region through which the lipophilic substrate may be transported; i.e. to act as a solvent for the hydrocarbon.

The carbon energy reserve metabolism for C. tropicalis growing on glucose and on hydrocarbon was investigated by Kaeppeli et al (118). They showed that glucogen was markedly accumulated in C. tropicalis growing on glucose, that the same effect was caused by a N- free medium and that the lipid contents did not show any significant change in either case. On the hydrocarbon substrate, lipid increased as substrate availability decreased whereas glucogen accumulation was only slight. However, the increase of lipid content on hydrocarbons did not reach the same level of accumulation as glycogen on glucose. In an N-free medium, both glycogen and lipids

were accumulated. From these results the authors suggest that glycogen is not substituted by lipids as carbon energy reserve on a hydrocarbon substrate.

The relationship between the function and structure of the n-alkane utilizing yeast cells were investigated by Hirai et al (119), Osumi et al (120, 121) and Teranishi et al (122, 123). They observed many interesting features of the physiological activity and the ultra structure of the cells; morphological change depending upon the chain length of n-alkane substrate.

All microorganisms which grow on aliphatic hydrocarbons as the sole carbon source incorporate into their lipids a large number of fatty acids formed as intermediates. Microorganisms cultivated on alkanes with an odd number of carbon atoms contain high amounts of corresponding odd carbon fatty acids in their lipids, whereas microorganisms cultivated on alkanes with an even number of carbon atoms posses high amounts of the even carbon fatty acids (125). Fritsche and Krieger (126) have compared the content of several enzymes associated with the carbon

metabolism in cells grown on glucose and on n-alkanes. In glucose grown cells there is a repression of the malate dehydrogenase and of the isocitrate lyase (enzymes involved in the catabolism of alkanes). On the other hand in alkanes grown cells there is a reduced level of enzymes for the glucose degradation or the glucose intermediary metabolism (Pyruvate Kinase and hexokinase). The authors suggest that the additional enzymes required for the alkane metabolism are synthesized at the expense of other enzy-mes.

Ermakova and Finogenova (127) found a higher activity of the glyoxylate shunt enzymes in alkane grown yeasts. It has been suggested that the glyoxylate cycle regenerates Acetyl Co A during alkanes oxidation.

A paper by Mallee and Blanch covers excellent review of the suggested mechanisms for the growth of microorganisms on hydrocarbons (123).

I. 2. 6. 5. SURVEY OF RESEARCH AND DEVELOPMENT ACTIVITIES ON SINGLE CELL PROTEINS PRODUCTION.

Single Cell Proteins refer to dried cells of groups of microorganism that have been considered for food or feed use, including algae, bacteria, yeasts molds and higher fungi (128).

People have eaten certain microorganisms as a portion of their diet since ancient times. Top fermenting yeast (Saccharomyces Species) was recovered as a leavening agents for bread as early as 2500 B.C. (129).

The current practices utilized in cultivating various species of Mushroms in China, Japan, Malaya and the Philippines originated from ancient lore. Blue green algae of the genus spirolina grown in alkaline lakes in certain regions of the world is considered as a good source of protein. A number of pond and tank systems for large scale algal cultivation have been developed in various countries including Japan, west Germany, India, Algeria and Mexico (130, 131, 132, 133). Purposeful cultivation of

microorganisms for direct use in human foods or animal feed is a fairly recent development. Baker's yeast (Saccharomyces cerevisiae) was grown in an aerated molasses ammonium salts medium for use in Germany during World War I. Aerobic yeasts particularly Candida utilis (torula yeast) were produced in Germany for food and feed use during World War II (135, 234). Numerous reviews and symposia describe the development of processes for utilizing different raw materials including simple sugar, starches, cellulose, agricultural and food processing wastes and hydrocarbons by bacteria, yeasts, molds and higher fungi (136, 137, 138, 139, 140, 141, 142, 143, 144, 145).

I.2.6.5.1. Bacteria and Actinomycetes :

I.2.6.5.1. A. Production

Bacteria are of interest for use in Singel Cell Proteins production because of their high growth rate (20 - 30 minutes generation time) as compared to 2 - 3 hours for yeast and 16 hours or more for algae, molds and

higher fungi. Various species of bacteria utilize a wide range of carbon and energy sources including those from renewable resources such as carbohydrates (Sugars, Starch, Cellulose) in either pure form or in agricultural and forest product wastes or those from nonrenewable resources such as hydrocarbons and petrochemicals.

A wide range of bacterial species has been considered for Single Cell Proteins production. Important consideration in selecting bacterial cultures suitable for use in Single Cell Proteins production include specific growth rate, yield on a given substrate, pH and temperature tolerance aeration requirements and genetic stability Table (1).

Substrate and nitrogen concentrations are important factors affecting growth of bacteria. Substrate concentration are in the 1-5% range but may be considerably lower for continuous cultures.

During growth pH is controlled in the range 5-7, (which is prefered by Bacteria) by the rate of ammonia or phosphoric acid addition to the medium. Temperature tolerance is an important characteristic of bacterial

Table (1).

Growth of Selected Bacteria on Hydrocarbon Substrate

Organism	Carbon Source	Temp. °C	pH	Specific growth rate μ_{-1} or D(h^{-1})	Culture density (g/L)	Yield (g/gsub)	References
Achromobacter delvacvate	Diesel Oil	35-36	7.0	---	10 - 15	---	(163)
Acinetobater (Micrococcus) cerificans	Gas Oil	30	7.0	0.4-1.0	8 - 10	0.10 - 0.12	(300)
	n - C_{16}	--	---	1.1-2.0	8 - 10	0.80 - 0.90	(300)
Methylococcus capsulatus	Methane	37	6.9	0.14	0.4	1.00 1.03	(167)
Norcardia sp.	N-alkan s	30	6.8	1.25	14.7	0.98	(302)
Pseudomonas sp.	Fuel Oil	36-38	7.0	0.16	16	1.00	(164)
Pseudomonas sp.	Methane	32	5.7	0.06(D)	0.8	0.99	(152)

strains for Single Cell Proteins production from hydrocarbons or alcohols. For example in the production of bacterial Single Cell Proteins from methane, heat production is in the order of 10 K Cal/gm of cells at a yield co-efficient of 1.0 gm of cells per gram of methane (146). Therefore cooling cost for bacterial Single Cell Proteins production can be significant unless high temperature tolerant strain in the range of 35° - 45° are used. Holve (147) and Wang et al (148) discuss methods for calculating the heat production associated with bacterial growth on hydrocarbon and carbohydrate substrates based on the elemental composition of the cells.

Oxygen transfer to growing cells is also an important factor in bacterial Single Cell Proteins production under aerobic conditions, especially from hydrocarbons, methanol or ethanol. Mateles (149) presented an empirical equation for estimating oxygen requirements for cell production based on the elemental compositions of the carbon source and cells, the molecular weight of the carbon source and the yield of cells based on the carbon source consumed.

Also cooling requirements can be gauged from the estimated oxygen requirement by use of the factor 0.11 K Cal/milli mole of oxygen consumed, developed by Cooney et al (150).

Maintaining sterility is important in the mass cultivation of bacteria. Undesirable contaminants such as enteric pathogens may grow equally as well as the desired strain since most bacterial Single Cell Proteins processes operate in a pH range of 5 to 7. Metabolic excretion products such as amino acid may provide sufficient nutrient for the growth of contaminants.

During 1960s there was considerable interest in the potential for producing Single Cell Protein from bacteria using gaseous and liquid hydrocarbon and chemicals derived from them as methanol and ethanol.

Research on a pilot plant scale for producing bacterial Single Cell Proteins from methane using Methylococous capsulatus (146, 151) or a mixed culture of Pseudomonas sp, Hyphomicrobium sp, Acinetobacter sp, and Flavobacterium sp (152, 153) has been conducted at Shell Research Limited in the United Kingdom. The productivity of the

processes for continuous production for bacterial Single Cell Proteins from methane is limited by transfer of oxygen and methane from the gas phase to the bacterial cells, occurence of simple or double gaseous substrate limitation, high heat production necessitating cooling and possible formation of inhibitory products (146, 154).

In addition potential explosive hazards require operation below 12.1% by volume of oxygen (146). Therefore it is expected that the capital costs for production of bacterial Single Cell Proteins from methane may be significantly higher than from other substrates.

Kyowa Hakko Kogyo Company Limited in Japan has developed several processes for the production of bacterial Single Cell Proteins from gaseous hydrocarbons. Methane, ethane, propane, n-butane or mixtures of these hydrocarbons were utilized by Brevibacterium ketoglutamicum ATCC No 15 587 (155). Other bacteria that have been investigated for growth on gaseous hydrocarbon substrate include various mixed cultures on methane (156, 157), Arthrobactre simplex on propane and butane (158) and

Carynebacterium hydrocarbonoclastus on propane (159). The actinomycete, Nocordia paraffnica utilizes n-butane readily. (160). Liquid n-alkanes are also suitable carbon and energy sources for a wide range of bacteria and actinomycetes.

Acinetobacter cerificans has been investigated on a small pilot plant scale for the production of Single Cell Proteins from purified normal alkanes by Exxon Corporation in a joint venture with Nestle Alimentana S.A. (161, 162). The Chinese Petroleum Corporation Taiwan has developed, on a pilot plant scale, process for producing Achromobacter delvacvate from diesel oil (163) and Pseudomonas No 5401 from fuel oil (164). However none of these processes has been practiced on a full commercial scale.

Actinomycetes, including Mycobacterium phlei and Norcadia sp, have been grown on liquid C_{10} - C_{20} hydrocarbons on a laboratory scale (165). However no further development of these processes has occured.

I.2.6.5.1. B. Yield :

A number of factors affect growth rate, yield, and productivity of bacteria used for Single Cell Proteins production. Oxygen transfer, mass transfer of substrate to the cell and heat production are important limiting factors for bacterial growth on hydrocarbons (as carbon and energy sources). Also inhibitory substances may be produced during growth particularly in continuous culture. As shown in the Table typical yields of bacteria and actinomycetes grown on hydrocarbons range from 0.80 to 1.20 gm dry matter/gm substrate utilized. The maximum theoretical yields of bacteria from methane assuming the involvement of a mixed function oxidase is 0.91 gm dry cells/gm substrate according to Van Dijker and Harder (166).

Harwood and Pirt (167) attained experimental yields for Methylococcus capsulates of 1.00 - 1.03 gm (dry Wt)/gm methane.

I.2.6.5.1. C. Cell Recovery :

A number of problems arise in recovering bacterial

cells from the growth medium. In most Single Cell Proteins processes, cell densities are in the order of 10-20 g/l (dry wt). Consequently large volumes of water must be handled. Also bacterial cells have a smaller size (1-2 um) than other microorganisms and bacterial cell densities are very close to that of water. Thus the cost of separating bacterial cells by centrifugation would be prohibitively high.

Wang (113, 114, 115) estimated that total costs for centrifugal separation of bacterial from the growth medium would be approximately four times as great as for yeasts.

Labuza (171) pointed out the plate and frame filter presses are not amenable to continuous processing as used in Single Cell Proteins production. Also in vaccum filters, the cell size and packing density of bacteria results in compression on screens and filteration ceases because the void volume decreases to nearly zero. The use of filter aids and flocculants would contaminate the cell product and make it undesirable for food or feed applications.

Acinetobacter cerificans can be concentrated by a two-zone froth flotation process according to Perkins and Fur long (161). Concentration of bacterial cells in the froth were significantly greater than those in the slurry.

Imperial Chemical Industries Ltd have developed a proprietary process for separating Methylophilus methylotrophus grown on methanol from the growth medium without using flocculating agents. A dewatered cell product 25 g/l (dry wt) or greater is then concentrated by conventional decanter - type centrifuges (173). The resulting concentrated product is then dried.

Hoechst/Uhde have operated a pilot plant scale process for separating Methylomonas clara grown on methanol from the culture medium based on electro chemical cogulation and centrifugation. The cell product is then spray-dried (174).

I. 2. 6. 5. 2. Yeasts

A. Production :

The technology of yeast production has developed

since the nineteenth century. Papers of champagnat (175), presented in the Sixth World Petroleum Congress on the possibility of utilizing the hydrocarbons of mineral oil as a carbon source for cultivating yeast gave a new impetus to scientific and industrial laboratories in many countries to take up materialization of this possibility and to elucidate the numerous problems associated with the conversion of hydrocarbon into a microbial mass. Table (2).lists some of the yeasts and sybstrates that are of potential interest for use in Single Cell Proteins production. Many of the characteristics cited for bacteria are important criteria in selecting yeasts for use in Single Cell Protein production, particularly specific growth rate, yield on a given substrate, pH and temperature tolerance, aeration requirements and genetic stability. Also the yeast strain must not be pathogenic to plants, animal or humans.

Substrate and nitrogen concentration for yeast growth should be adjusted to provide a C : N ratio in the range of 7 : 1 to 10 : 1 to favour high protein content. Concentration of hydrocarbons in batch cultures ranges from

Table (2)

Growth of Selected Yeasts on Hydrocarbon Substrate

Organism	Carbon Source	Temp. °C	pH	Specific growth rate μ_{-1} or D(h^{-1})	Culture density (g/L)	Yield (g/g_{sub})	References
Candida lipolytica	N.alkanes	32	5.5	0.16(D)	23.6	0.88	(303)
	Gas Oil	30	4.0	---	25	0.18	(221)
Candida tropicalis	N.alkanes	30	3.0	0.15-0.24	10-30	1.0-1.1	(304)
Hansenula polymorpha	Methanol	37-42	4.5-5.5	0.22 0.13 D	1.2	0.36	(179)

1 to 5%. In continuous cultures with hydrocarbon or alcohols lower concentration are used. At higher C : N ratio many yeast particularly those of the genus Rhodotorula accumulate a substantial portion of the cell weight in the form of lipids. Anhydrous ammonia or ammonium salt are suitable nitrogen sources for yeast of interest in Single Cell Proteins production.

Anhydrous ammonia can be used in combination with phosphoric acid to effect pH control. It is desirable to select a yeast having a high specific growth rate and yield in the pH range of 3.5 - 4.5 to minimise the possibility of growth of any bacterial contaminants.

As with bacteria heat is liberated by yeasts growing on carbohydrates, hydrocarbons or alcohols. For candida species growing on hydrocarbons calculted value of heat liberated range from 4400 - 8000 K Cal/Kg for yields of 1.2 - 0.9 gm cells (dry wt)/gm substrate utilized, respectively (176, 177). Actual observed values of heat liberated during growth are in this range. A value of 7600 K Cal/Kg dry weight of yeast is cited by Bennett et al (178) for Candida sp. growing on n-alkanes.

It is clear that heat tolerance is a desirable factor for a yeast to be used in Single Cell Proteins production. Most yeasts have the highest specific growth rate in the range 30 - 40 °C. A yeast capable of growing well in the range 40-45 °C would be of considerable interest. Hansenula polymorpha ATCC 26012 has an optimum growth temperature range of 37 - 42°C (179).

The growth rate of yeasts under aerobic conditions depends upon the rate of mass transfer of oxygen and substrates to and across the cell surface. For growth on carbohydrates the oxygen requirement is 1 g or less per g (dry wt) of cells (180). On n-alkanes the oxygen requirement is approximately 2 gram of oxygen per gram (dry weight) of cell (181). Candida lypolytica was grown in a simple medium with dodecane as sole carbon source under batch and continuous fermentation condition. The oxygen demand of the cells and the effect of operating condition on cell growth were evaluated experimentally (182).

According to Laine and du Chauffant(177) yeast grown on hydrocarbons require an oxygen transfer

co-efficient of 10-15 Kg/m^3. h. A Waldhof type agitated and aerated fermentor has been used extensively for achieving desired levels of aeration in food and fodder yeast production from both carbohydrates and hydrocarbon substrates (172). In recent years, air-lift type fermentors of the type developed by Lefrancois (183) have been used to an increasing extent to achieve increased oxygen transfer rates and/or decreased power requirements for aeration and agitation, particularly in the production of yeast Single Cell Proteins from hydrocarbons (184, 185, 186).

For the economic manufacture of a mass product by a microbial process as for example Single Cell Proteins it is important to chose a reactor that meet the following requirements.

- large mass transfer rate for oxygen and substrate at low energy input.
- the reactor must be capable of being constructed and operated in large units.
- a simple and robust design which is characterized by

low construction in costs, which is easy to keep under sterile condition has low maintanence costs and a high on stream availability.

The loop reactor with jet drive meets these requirements. Though the development of this type of bio reactor is still in its infancy, its suitability has already been proved (187).

Yeast Single Cell Proteins production may not need strict under sterile conditions. In either batch or long term continuous yeast production one must balance the need for contamination control, by maintaining sterile condition with the capital and operating costs of the equipment required.

In producing C. utilis (torula yeast) from sulfite waste liquor, the medium is passed through heat exchangers to effect sterilization and then charged into clean Waldlof-type fermentors. However sterile condition are not maintained during growth and contamination control depend upon maintaining a pH below 6.0, aerating with a sterile air

supply, and maintaining a large population of yeast in the fermentor (188, 189).

In the case of yeasts grown on hydrocarbons, clean but non-sterile conditions were used by British Petroleum Company Ltd., in an air-lift fermentor pilot plant for producing Candida sp. from gas oil at Lavera, France. However in producing Candida sp.from purified n-alkanes in a conventional agitated-aerated fermentor at Grangemouth, Scotland, British Petroleum used a completely sterile system and maintained sterile conditions throughout the operations (190, 191).

I.2.6.5.2. B. Yield :

Factors affecting the growth rate, yield and productivity of yeasts used for Single Cell Proteins production from various substrates are similar to those for bacteria. Experimental yields on carbohydrates range from 0.39 to 0.55 gm cells (dry wt)/gm. substrate utilized. These values are similar to the 0.51 gm (dry wt) / gm. substrated reported by Hernandez and Hohnson (192) for Candida utilis growth on glucose.

The yield of various Candida species grown on purified n-alkanes range from 0.88 to 1.1 gm (dry wt) / gm substrate. These yields were obtained in pilot plant scale operation are representative of values attainable in large scale oproduction facilities. These values agree with the calculation of Guenther (193), which indicate that yields greater than 1.0 gm (dry wt) / gm substrate are theoretically possible from hydrocarbon on an energy basis.

Various equation have been developed for showing the stoichometry of yeast growth on carbohydrates or hydrocarbons (178, 186, 193, 194). Typical equations are given by Bennett et al (178) for carbohydrate and hydrocarbons.

1. $8n\ CH_2O + 0.8n\ O_2 + 0.19n\ NH_4^+ + \text{essential elements} \longrightarrow$

$n(CH_{1.7}O_{0.5}N_{0.19}\ \text{Ash}) + 0.8n\ Co_2 + 1.3n\ H_2O + 80000^n K\ \text{cal}$

$2n\ CH_2 + 2n\ O_2 + 0.19n\ NH_4 + \text{essential elements} \longrightarrow$

$n(CH_{1.7}O_{0.5}N_{0.19}\ \text{Ash}) + n\ Co_2 + 1.5\ H_2O + 200000n\ K\ \text{cal.}$

The essential differences to note are that the use of hydrocarbon substrates instead of carbohydrates, require the supply of about two and a half times as much atmospheric oxygen and releases over twice as much heat in the reaction. In fermentations such as yeast propagation, it is considered a reasonable assumption where de Beeze and Liebmann (197) consider aeration the most important factor accounting for 10 - 20 % of the total production cost. Darlinton (194) examined the oxygen requirements of a hydrocarbon fermentation directed as yeast production. For his calculation he assumed.

1. The composition of the product from the carbohydrate and hydrocarbon fermentation is identical.
2. The typical microbial composition is 47% carbon, C; 6.5% hydrogen, H; 7.5% nitrogen, N; 8% ash and 31% oxygen, O; by difference.
3. The dry weight yield of yeast from carbohydrate is 50% (140).
4. The dry weight yield of yeast from hydrocarbon is 100% (141, 173).

With this assumption the empirical formula (C, H, O formula) for 100 g . Yeast of this composition would be $C_{3.92}H_{6.5}O_{1.94}$ and the amount of carbohydrate (CH_2O) required based on a 50% yield would be 200 g or 6.67 g. moles CH_2O.

$$6.67\ CH_2O \longrightarrow C_{3.92}H_{6.5}O_{1.94}$$

$$\text{i.e. } 6.67\ CH_2O + 2.1O_2 \longrightarrow C_{3.92}H_{6.5}O_{1.94} + 2.75\ Co_2 + 3.42\ H_2O$$

i.e the production of 100 g. dry yeast from carbohydrate requires 67.2 g oxygen considering the hydrocarbon fermentation in the same way the production of 100 g. dry yeast at a 100% conversion requires 100 g. hydrocarbon (CH_2)

$$7.14\ CH_2 \longrightarrow C_{3.92}H_{6.5}O_{1.94}$$

$$\text{i.e. } 7.14\ CH_2 + 6.135\ O_2 \longrightarrow C_{3.92}H_{6.5}O_{1.94} + 3.22CO_2 + 3.89\ H_2O$$

i.e the production of 100 g. dry yeast from hydrocarbon requires 196.32 oxygen. Thus the oxygen requirements of a hydrocarbon yeast fermentation is almost triple that of a yeast carbohydrate fermentation producing an equal amount of product. If oxygen transfer is the limiting

factor in yeast propagation, then a hydrocarbon fermentation is doomed to a lower production rate than that of the corresponding carbohydrate fermentation.

It is also apparent that less hydrocarbon is required to produce a given amount of product.

The above mentioned equations vary with the composition of the yeast cell product and the same consideration apply to other microorganisms used for SCP production, including algae, bacteria, molds or fungi. Holve (147) presented a series of equations for describing the stochiomentry of conversion of carbohydrate, hydrocarbons, alcohols or organic acids to Single Cell Proteins by microorganisms.

I. 2.6.5.2. C. Cell Recovery :

Yeast cell range in size from 5 to 8 um and have a density of 1.04 - 1.09 gms/cm^3. They can be recovered readily from the growth medium by continuous centrifugation. Usually the cells are centrifuged in a first stage to initially dewater, yielding a yeast stream. This is

followed by two subsequent washing centrifugations. The final washed yeast cream usually contains 15 - 20% solids. This procedure is typical of that used in the recovery of C. utilis after growth on sulfite waste liquor (133, 198).

In the case of Candida sp. grown on n-alkanes, a wash with surfactant may be required to remove traces of hydrocarbons following initial centrifugation (168, 171). With gas oil, a much more complex separation and clean up procedure must be used to remove residual hydrocarbons and lipids. Decantation, phase separation with solvents, washing with surfactants and solvent extraction have been used alone or in combination (199).

Also hydrocarbons and lipids can be depleted by allowing the yeast to remain in contract with the growth medium in the absence of added substrate for varying periods (141, 200).

Wang (169, 170) has analyzed methods and costs for recovering microbial cells including yeasts. He pointed out that the cost of cell separation could be reduced if

the size of the cell could be increased or if flocculants compatiable with a food or feed grade product could be developed.

The separated cells can either be drum or spray dried. According to Labuza (171) drum drying brings about two to five logarithmic cycles of kill of yeast cells. Associated bacteria having similar thermal resistances would be killed to the same extent. However drum drying if not carefully controlled may result in excessive darkening of the product. Under the temperature and time conditions used in spray drying, 5 - 8 logarithmic cycles of kill of yeast are obtained and cells of pathogenic bacteria such a Salmonella and Staphylococcus should be killed to the same extent. The colour and furnished characteristia of the final yeast produced prepared by spray-drying are superior to those obtained after drum drying (171).

I.2.6.5.3. Nutritional Value of Single Cell Protein :

There is considerable information available on the composition of microbial cells, including protein, amino acid, vitamin and mineral contents (200). Table No. (3 A) summarizes proximate analysis of selected microorganisms of interest in SCP production. Crude protein value are based on nitrogen values multiplied by the factor 6.25. Protein content computed in this manner is used for formulating protein product into feeds. However, these values do not reflect the true protein content of microorganisms since nitrogen, or non protein nitrogenous substrates, including nucleic acid is erroneously included as protein.

Protein and lipid contents of microorganisms reflect the composition of the medium and growth condition. Yeast mold and higher fungi have higher cellular lipid contents and lower nitrogen (and protein contents) when grown in media and a deficiency of nitrogen. Ash contents of SCP product also vary depending upon the mineral content of the growth medium.

Table (3 A)

Proximate Composition of Selected Organisms of Interest For SCP Production.

	Bacteria	Yeast	
	Acinetobacter (Micrococcus) *cerificans*	*Candida lipolytica* (Toprina)	*Candida lipolytica* (Toprina)
C.Substrate	Hexadecane	N-alkanes	Gas oil
Nitrogen g/100 g. dry wt.	11	10	11
Protein.g/100 g. dry wt.	72	65	69
Fat.g/100 g. dry wt.	--	8.1	1.5
Ash.g/100 g. dry wt.	--	6	8
References	(162)	(201)	(201)

Table (3 B) presents amino acid contents of selected microorganisms of interest in SCP production as compared with the Food and Agricultural Organisation (FAO) reference values. In general only a few microorganisms, particularly the bacteria, have amino acid profiles that compare favourably with the FAO values as regards methionine content. With yeast in addition to methionine deficiencies lysine/arginine ratios are wider than desirable and must be adjusted in poultry rations (201).

However, actual performance of SCP products as determined in feeding studies is the most important measure of nutritional value. For human food applications, protein digestibility, and Protein Efficiency Ratio (PER), Biological Value (BV) or Net Protein Utilization (NPU) determined in rats are used as measures of the utility of an SCP product. For animal feed applications metabolizable energy, protein digestibility and feed conversion ratio are usual measures of performance in broiler chickens, swine and calves (201, 202) and laying hens (203, 204).

Table (3 B)

Amino Acid Content of Selected Organisms for SCP Production

Organism	Substrate	Lys	Phe	Lev	Lie	Val	Try	Thr	Met+Cys	Ref.
		(g/16g N.)								
Acinetobacter (Micrococcus) cerificans	n.alkanes	5.2	3.6	6.5	4.3	5.4	1.3	4.6	1.9	(162)
Methylococcus capsulatus	Methane	5.7	4.6	8.1	4.3	6.5	---	4.6	3.3	(146)
Yeast Candida lipolytica	n.alkanes	7.0	4.4	7.0	4.5	5.4	1.4	4.9	2.9	(222)
Candida lipolytica	gas oil	7.8	4.8	7.8	5.3	5.8	1.3	5.4	2.5	(222)
Candida tropicalis IIP - 4	n.alkanes	7.5	5.3	6.8	3.0	2.7	1.2	5.2	2.1	
FAO reference		4.2	2.8	4.8	4.2	4.2	1.4	---	2.2	

Lys: Lysine; Phe: Phenylalanine; Lev: Leucine; Lie: Isoleucine; Val: Valine
Try: Tryptophane; Thr: Threonine; Met: Methionine; Cys: Cystine.

Table (4) shows typical values obtained from animal feeding studies of SCP product. Supplementation with methrinine is necessary to obtain a PER or BV equivalent to animal protein such as casein, for most microorganisms (201, 205).

The results of feeding studies with broiler chickens, using Methylophilus methylotrophus (206) do not show significant differences between experimental and control diets at the 10% level of SCP in the diet. Similar results were also observed with broiler chickens in feeding studies with the yeast C. lipolytica (201) and C. tropicalis (207) up to the 15% level in the diet. In swine feeding studies with yeast grown on n-alkanes feed conversion ratio tend to decrease as percentage of SCP in the diet increase above about 10% (141). When yeast SCP grown on hydrocarbon is used as a milk replacer for calves and steers, the limit appears to be approximately 7.5% in the ration (201).

In the United States, the Food and Drug Administration regulation allow the use of the dried yeasts S. cerevisiae,

Table (4)

Performance of Selected Single Cell Protein Products in Animal Feeding Studies.

Single Cell Protein and Substrate	Treatment and % in diet.	Animal	Performance: Protein digestibility %	Performance: Biological Value (BV)	References
Bacteria *Acinetobacter* (Micrococcus) *cerificans* n.hexadecane	Dried	Rat	83.4	67	(301)
Yeast *Candida lypolytica* (n.alkanes)	(1) Dried	Rat	96.0	61	(201)
	(2) Dried + 0.3 (3) Dl-methionine	Rat	96	91	
	(4) Dried; 10%	Broiler chicken	88	--	
	(5) Dried; 7.5%	Pig	92	--	
Candida lipolytica (gas oil)	Dried	Rat	94	54	(201)
	Dried + 0.3 Dl.methionine	Rat	95	96	

and C. utilis in foods provided that the folic acid content of the yeast does not exceed 0.04 mg./gm. Use of dried yeast in food has been limited to flavour enhancement and vitamin B supplementation. High levels of C. utilis in foods have led to poor acceptance. Bressani (208) discussed earlier studies on supplementation of foods with yeast and on their value in human nutrition. Young and Scrimshaw (209) pointed out that yeast and algae have low protein digestibility value that can be improved considerably if these SCP products are processed suitably. However nucleic acid content is a limiting factor in the extent to which SCP product can be used in human foods.

Safety :

There are several key problem areas in using SCP product for human foods. There include the following :

(1) High nucleic acid content of many microorganisms that would result in kidney stone formation or gout if consumed on a significant portion of the protein intake.

(2) Poor digestibility and possible adverse gastrointestinal and skin reactions and

(3) Possible presence of toxic or carcirogenic compounds from residues of substrate, from biosynthesis by the organisms, or from chemical reaction during processing and drying.

Calloway (210) pointed out that adverse responses may be observed in human subject fed with bacteria, algae or yeast SCP products even though favourable results were obtained previously in animal studies. Published values of nucleic acid content on a dry weight basis of bacteria are upto 16% (211, 206) and that of yeast are 6 - 11 % (212, 213). Scraimshaw (214) states that a human intake of 2 gm of yeast nucleic acid per day would be within safe limits.

From the standpoint of animal feeding, high nucleic acid content in SCP are not a problem with ruminants and are better tolerated by nonruminant livestock than by humans. De Groot (215) discusses minimal evaluations for establishing the safety of SCP. Taylor et al (216) presents criteria for the evaluation of SCP for feeding nonruminants.

The possible hazards from the presence of traces of residual n-alkanes, odd-carbon fatty-acid, or polycyclic aromatic hydrocarbons in SCP grown on hydrocarbon substrates were analysed. The results of extensive studies have shown that the polycyclic hydrocarbon content of Candida sp. grown on hydrocarbons were no greater than those on other commercial yeast products or other representatives foods. Also no carcinogenic, mutagenic or teratogenic effects were noted in appropriately designed studies (217). Table (5).

The PAG Ad Hoc working Group on Single Cell Proteins concluded that low level of residual n-alkanes and presence of odd-carbon fatty-acids do not consitute a hazard (218, 219).

Table (5)

Summary of Toxicological Experiments with Toprina (B.P. SCP)

Type of experiment	Duration	Animal	Dietary level	Results
Acute toxicity	6 weeks	Rat	40	No difference between control and test groups
Subchronic toxicity	90 days	Rat	10,20,30	No evidence of abnormalities
Chronic toxicity	2 years	Rat	10,20,30	No evidence of abnormalities
Carcinogenicity	2 years	Rat	10,20,30	No evidence of carcinogenic effect
Multiple generation studies	15 generate	Rat	10,20,30	No evidence of teratogenic mutagenic effect
	23 generate	Japanese guail	10,20,30	No effects on reproduction fertility or physical characteristic
Teratogenicity	Pregnancy	Rat	10,20,30	No teratogenic effect
Mutagenicity	5 day feeding period Males	Rat	60	No mutagenic effect
Induced toxicity in products from pig poultry fed on alkane yeast		Rat	various	No different effects from products of similar animals on yeast free diets
Sensitisation - injection - intracutaneous - palich	2 weeks feeding	Rat guinea pig pig monkey	10	No sensitivity reaction in any of the species

I. 2.6.6. Large Scale Fermentation Studies Step Towards Industrialization

During the early 1960's British Petroleum Company Limited introduced the concept of using the microorganism Yeast - C. lipolytica for the production of SCP from hydrocarbon fractions including gas oil and purified n-alkanes prepared by molecular sieve adsorbtion method (220, 221, 222, 223, 224, 225). In the case of gas oil the objective was to utilize the n-paraffinic hydrocarbon to effect dewaxing and concurrently to produce SCP. About 10% of the gas oil is utilized by the yeast and the remaining 90% is returned to refinery. British Petroleum constructed two pilot plant for producing Single Cell Proteins from hydrocarbon. A 16000 metric ton/year plant at Lavera, France was designed to utilize gas oil containing 25% of C_{15} - C_{30} n-paraffins, having a boiling range of 300 - 400°C. A continuous air lift fermentor was used under clean but non-sterile operating condition. A 4000 metric ton/year pilot plant has been constructed and operated at Grangemouth, Scotland. It utilized purified n-alkanes prepared by molecular sieve process.

A continuous aerated and agitated fermentor was operated under aseptic condition. The mineral nutrients were sterilized by heating, while air and ammonia are sterilized by filteration. (222, 226).

The British Petroleum processes led to the development of similar yeast based SCP processes utilizing hydrocarbon substrate throughout the world.

A 100,000 metric ton/year SCP plant constructed in Sardinia by British Petroleum and Ente Nationale Idro Carbuni has not yet been placed into operation because of a dispute with Italian Governmental authorities over hydrocarbon residues and product quality (227, 231, 232). Another 100,000 metric ton yeast SCP plant constructed by Liquichimica Biosintesi S.P. at Saline, di Montebello Italy, using the Kanegafuchi Chemical Industry process has not been operated for the same reason, (231, 232).

The agricultural division of Imperial Chemical Industries (ICI) has evolved a method of protein production using methanol produced from natural gas as the feed stock and employing a bacterium as the process organism

and the final product is trade named " PRUTEEN". A 1000 tonnes a year pilot plant incorporating ICI's own fermentation system has been in operation since 1974 and based on their successful trials, they have set up 60,000 T/A commercial unit with an investment of nearly 40 million pounds. The organism used is Methlophilus (Pseudomonas) methylotrophus (228, 229, 233). Gow et al (206) have described the novel pressure cycle airlift fermentor used in this process.

A consortium of Hoechst Uhde and Gelserberg has been engaged in a joint project of development of Single Cell Proteins process on methanol and 1000 T metric tons/ year unit has been constructed near Frankfurt West Germany (234). Apart from the protein market Hoechst expects to derive considerable benefits from secondary metabolities obtained as co-product of SCP plant.

In Eastern Europe where the drive towards proteins self sufficiently is stronger the industry for making SCP has been making rapid strides over the last five years. Industrial yeast production is mostly based on n-paraffins,

but agricultural wastes and by-product of wood processing and cellulose industries are also used as substrate for fermentation. This industry is said to have grown by 60 percent during 1975-80 and is expected to grow further. There appears to be two types of Soviets Bio-proteins production; one a low grade material based on carbohydrate raw material and the other, a higher grade protein - Vitamin concentrate predominantly based on n-paraffins. The average protein contents of the first type is said to be around 53% whereas of the second type is around 60%. Paraffins based plants producing the more concentrated material are located in Bashkiria, Polotsk, Krishi, Kstowo Svetloyarsk, Angarsk and at Kremenchug. A 300,000 T/year n.paraffins based unit is planned at Mozyr as a joint ventrue of all the East European countries (235).

Raniprot, which is a Romanian-Japanes joint venture Company, has started the 60,000 T/year commercial production of Single Cell Protein from n-paraffins in the Curtea de Arges town around 130 km from Bucharest. They have

emphasized that economic situation of SCP commercial production is not so easy due to the general permanant trend of crude oil price increase. Even under such circumstances the necessity of having SCP plant has been felt due to greater demand for food and in particular for meat production. Viton (Ronipron) is the registered trade mark of Dinippon Ink and Chemicals SCP animal feed. A variety of tests to affirm the safety and nutritional value of Viton have been done not only in Japan, but also in INRA, France ILOB in Netherlands, University of Munich Celk and Volken rode, in West Germany.

Shanghai Institute of Organic Chemistry, China has been operating 100 tons per year pilot plant using an yeast strain belonging to the species Candida tropicalis and has successfully completed long term and multiple generation toxicological studies on different species of animals.

The AMOCO Company USA has done considerable work using ethanol as substrate and their plant which is designed to produce up to 15 million pounds per year of

product trade named " TORUTEEN " is already in operation. It uses Candida utilis as the microorganism and the product is being sold in United States as nutritional supplements and flavour enhancements for such processed foods as meat patties, baked goods, frozen pizzas and sauces.

Phillips Petroleum, the big American oil Company is to produce Single Cell Protein from Alcohol for animal and human consumption. Phillips has chosen a yeast for its Single Cell Food. This has both nutritional and physiological advantages over bacteria for human consumption, according to Phillips Biotechnology Director Dr.John Norell, Although Phillips is test marketing its Single Cell Protein called " PROVESTEEN " as premium quality animal feed like Pruteen of ICI, its long term interests is in the far more lucrative market for human consumption. Dr. Norell sees " Provesteen " as a protein supplements for people for example as an additive to flour before baking or to rice in protein poor third world countries, rather than as a food on its own right (236).

Single Cell Protein Research based on sp. fossil sources especially normal paraffins became relatively

popular in Japanes business and university laboratories, only in the first half of the 1961, even though the commercial production of microbial protein using sulfite liquor as the source of carbon began in 1957. Japanese fermentation companies, chemical companies and oil companies began to study the commercialization of SCP production using n-paraffins. At the end of 1972 Kanegafuchi Chemical Industry and Dainippon Ink & Chemicals, the leaders in the commercialization of SCP in Japan, completed the design of 60,000 T/year n-paraffin based SCP production plants. However due to sudden change in the atmosphere of Japanese Society, the two companies stopped commercialization plans during February 1973. There after both companies exported the technology and set up overseas joint ventures, Kanegafuchi in Italy and Dainippon in Rumania. Studies in the commercialization of Single Cell Protein production using methanol as carbon substrate have been made by the Mitsubishi Gas and Chemical Co. In 1974 this company constructed a pilot plant with a capacity of 500 tons per year and in 1979 completed feeding trials of the Single

Cell Protein produced at this plant. The design and construction with a capacity of one hundred tone per year is under consideration.

In India, Indian Institute of Petroleum (IIP) Dehradun and Regional Research Laboratory (RRL) jointly entered into collaboration with Institute Français du Petrole, France during 1966. IIP based on its successful results obtained at its laboratories, at Dehradun, in agreement with Indian Oil Corporation (IOC) has set up research and development facilities in Gujarat Refinery premises with a view to conduct pilot plant studies and to produce Single Cell Proteins product in bulk quantities for acceptability tests and large scale field trial experiments. IIP has further collaboration with Central Food Technological Research Institute (CFTRI) Mysore for Nutritional studies and for Toxicological Experiments with Industrial Toxicological Research Centre (ITRC) Lucknow. IIP also established contacts with Indian Veterinary Research Institute, Izatnagar, Gujarat Agricultural, Anand and National Dairy Research Institute, Karnal for large scale field trial experiments on farm animals.

I. 3. PRESENT WORK AND ITS SCOPE

Among the different uses of n-paraffinic hydrocarbons, those under oxidation, chlorination, nitration, sulphochlorination and sulphoxidation are quite old and enough work has already been done, since the beginning of this century, which is evident from the literature survey made and presented. However utilization of n-paraffinic hydrocarbons by fermentation to produce Single Cell Proteins is of recent origin and still in infant stage. Even though big oil companies all over the world made high investments in conducting industrial research and in setting up pilot plants, very few commercial units are in operation and some confusion exists among the policy makers regarding the acceptance of the product.

India with its human and animal population still growing at nearly 2% will face serious problems of supplying food and feed unless sufficient steps are taken to produce through unconventional sources.

Petroleum oil is a very precious commodity and today about 95% of the annual petroleum and natural gas

production is still used for power generation and the change to higher level of exploitation is only in its initial stage.

Crude oil production in India has steadily increased from 0.45 million tonnes in 1960 to nearly 30 million tonnes during 86-87 and nearly 45 million tonnes has been processed during 86-87. For the production of Single Cell Proteins it needs less than 5% of the total crude oil.

The present thesis covers the following different aspects involved in the development of the process and popularization of the product :

- Adaptation of an yeast strain to assimilate and grow on hydrocarbons.
- Selection of suitable hydrocarbon cut for Single Cell Protein production.
- Optimization of temperature, pH, aeration, agitation etc on the growth of yeast strain.
- Continuous fermentation studies to check the stability of the yeast strain for long duration fermentation

studies and to check the variation of cell concentration, productivity, protein content, lipid content at different dilution rates.

- Microbial dewaxing studies of kerosine fractions.
- Bench and pilot scale fermentation studies using pure n-paraffinic hydrocarbons and production of biomass.
- Treatment of biomass of get Single Cell Protein in pure powder form.
- Acceptability test results which include :
    - Nutritional studies carried out at Central Food Technological Research Institute - Mysore.
    - Toxicological studies carried out at Industrial Toxicological Research Centre - Lucknow.
- Summary of large field trial experiments conducted on farm animals at :
    - Indian Veterinary Research Institute -Izatnagar,
    - Gujarat Agricultural University - Anand and
    - National Dairy Research Institute - Karnal.