

SINGLE CELL PROTEINS

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YEAR : AUGUST 2009

STUDENT NUMBER:GS/MSc/Food/3631/08

Summery

This report gives you an idea what is Single cell proteins (SCP) and its history. Furthermore it gives the production process in detail. It contain various production processors such as BP process, ICI process, Bel Fromageries process, RHM Mycoprotein process.

In addition this give potential resources that can be used for single cell protein production. Furthermore this report gives criteria that you should consider when selecting mixture of microorganisms to your fomenter in SCP production process.

Further this briefly explains how the economic factor affects the production process. Further it gives advantages of SCP production process and advantages of SCP in general and vise versa. Furthermore it gives a brief account on quality and safety of SCP. Composition of single cell proteins are also given for further knowledge.

Key words : *Hygrogenomonas*, *Cellulomonas*, *Pseudomonas*, *Saccharomyces cerevisiae*

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INTRODUCTION

Most of the developing countries of the world have been facing malnutrition problem. The deficiency of protein in human food and animal feed is well recognized due to the rapid growth of population. It has been reported that in Pakistan the protein gap would continue to increase unless well planned measures are adopted to handle the situation. It is therefore, important to increase protein production by utilizing all the available ways and means. The increasing world demand for food and feed protein spurred the search for non-conventional protein sources to supplement the available protein source. A great deal of interest has been focused on the potential of agricultural wastes to microbial protein or single cell protein. The impetus behind single cell protein production lies partly in the need for more protein and partly in the commercial increase in the economic advantages gained by substitution of microbial protein for the conventional protein supplements used in livestock feeding.

single cell protein (SCP) typically refers to sources of mixed protein extracted from pure or mixed cultures of algae, yeasts, fungi or bacteria (grown on agricultural wastes) used as a substitute for protein-rich foods, in human and animal feeds. The term single cell protein (SCP) was coined at Massachusetts Institute of Technology (MIT) by a group of scientists in 1966. The term, as used today, is rather misleading, since it refers to not only the isolated cell protein but to "any microbial biomass from either uni and multicellular bacteria, yeasts, filamentous fungi or algae which can be used as food or feed additives". On an average, the microbial biomass contains about 45 to 55% protein, although in some bacteria the protein content is as high as 80%. The biomass also contains other essential nutrients as well, and as such it is an ideal supplement to conventional food supply.

Dried food-grade yeasts and their autolysates have been used for many years. Dried mycelia of mushrooms, by virtue of their pleasant flavour can be used in soups, sauces or gravy formulations.

History

Since 2500 BC yeasts have been used in bread and beverage production. In 1781 processes for preparing highly concentrated forms of yeast were established. In 1919 *Endomyces vernalis* yielded fats from sulphite liquor (from paper manufacture), and similarly in 1941 employing *Geotrichum*. The term SCP was coined in 1966 by Carol L. Wilson.

SCP Production Process

Single cell proteins develop when microbes ferment waste materials (including wood, straw, cannery and food processing wastes, residues from alcohol production, hydrocarbons, or human and animal excreta). The problem with extracting single cell proteins from the wastes is the dilution and cost. They are found in very low concentrations, usually less than 5%. Engineers have developed ways to increase the concentrations including centrifugation, flotation, precipitation, coagulation and filtration, or the use of semi-permeable membranes.

The single cell protein needs to be dehydrated to approximately 10% moisture content and/or acidified to aid in storage and prevent spoilage. The methods to increase the concentrations to adequate levels, and de-watering process require equipment that is expensive and not always suitable for small-scale operations. It is economically prudent to feed the product locally and shortly after it is produced.

Biomass and Single Cell Protein

Microbial cells are produced for two main applications,

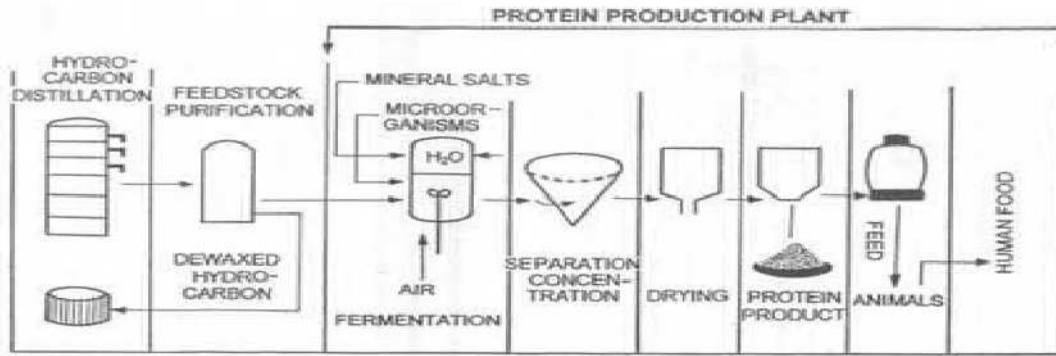
(a) as a source of protein for animal or human food, (Single Cell Protein) or (b) for use as a commercial inoculum in food fermentations and for agriculture and waste treatment. As a commodity, SCP must be competitive with commercial animal and plant proteins, in terms of price and nutritional value and must conform with human and animal food safety requirements. Productivity, yield and selling price are the major factors affecting the economics of SCP production. Microbial inoculants, which are used as a process aid, generally have a higher value. In this case, the objective of the production process is to optimize yield of viable cells of defined biological activity with good shelf life characteristics. *Saccharomyces cerevisiae*, is categorised primarily as a microbial inoculant. Inactive dried brewer's or baker's yeast is also used as a dietary source of vitamins and trace minerals in specific medical conditions. Considerable amounts of yeast extract are produced from bakers yeast as a source of flavour and vitamins.

Production

Large scale fermenters are required. High biomass productivity requires high oxygen transfer rates which promotes high respiration rates which in turn increase metabolic heat production and the need for an efficient cooling system. In order to maximise fermentation productivity it is essential to operate continuous fermentation processes.

Different processes have adopted different fermenter designs with respect to process requirements.

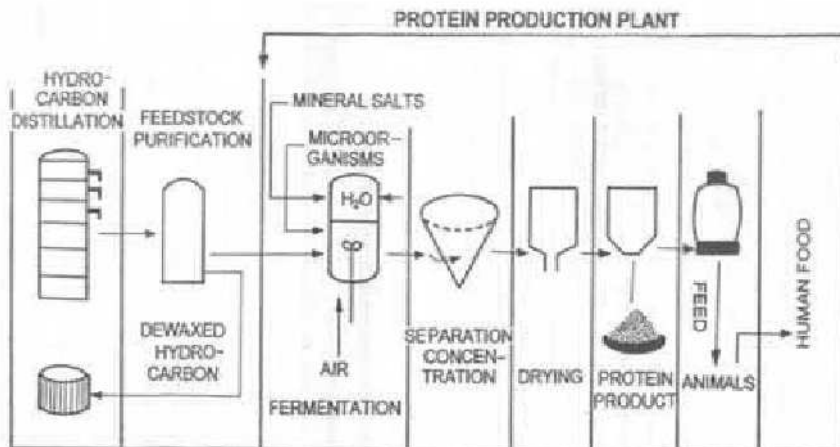
Diagrammatic Representation of Commercial Production of Barker's Yeast



Commerical Production

The raw materials that can be used for single-cell protein manufacture include whey, sulphate waste liquors, hydrocarbon waste from the pet petroleum industry, and the vats used to produce alcoholic beverages. The production process involves growth of the organisms in large fermenting tanks with forced aeration for vigorous cell-growth. Manufacture process used by British Petroleum Industry for single-cell protein from hydrocarbons is represented in figure below

Diagrammatic Representation of Commercial Production of Single Cell from Hydrocarons



Yeast, fungi, bacteria, and algae are grown on hydrocarbon wastes, and cells are harvested as sources of protein. It has been calculated that 100 lbs of yeast will produce 250 tons of proteins in 24 hours, whereas a 1000 lbs steer will synthesize only 1 lb of protein 24 hours

and this after consuming 12 to 20 lbs of plant proteins. Similar, algae grown in ponds can produce 20 tons (dry weight) of protein, per acre, per year

This yield is 10 to 15 times higher than soybean and 25 to 50 times higher than corn. There are both advantages and disadvantages in using microorganisms for animal or human consumption. Bacteria are usually high in protein (50 to 80 percent) and have a rapid growth rate. The principal disadvantages are as follows:

1. Bacterial cells have small size and low density, which makes harvesting from the fermented medium difficult and costly.
2. Bacterial cells have high nucleic acid content relative to yeast and fungi. This can be detrimental to human beings, tending to increase the uric acid level in blood. This may cause uric acid poisoning or gout. To decrease the nucleic acid level, an additional processing step has to be introduced, and this increases the cost.
3. The general public thinking is that all bacteria are harmful and produce disease. An extensive education programme is required to remove this misconception and to make the public accept bacterial protein.

Yeasts have as advantages their larger size (easier to harvest), lower nucleic acid content, high lysine content and ability to grow at acid pH. However the most important advantage is familiarity and acceptability because of the long history of its use in traditional fermentations. Disadvantages include lower growth rates, lower protein content (45 to 65 per cent), and lower methionine content than in bacteria.

Filamentous fungi have advantages in ease of harvesting, but have their limitations in lower growth rates, lower protein content, and acceptability. Algae have disadvantages of having cellulosic cell walls which are not digested by human beings. Secondly, they also concentrate heavy metals.

Single cell protein basically comprises proteins, fats, carbohydrates, ash ingredients, water, and other elements such as phosphorus and Potassium. The composition depends upon the organism and the substrate which it grows. Some typical compositions which are compared with soymeal and fish meal. If SCP is to be used successfully, there are five main criteria to be satisfied;

1. The SCP must be safe to eat.
2. The nutritional value dependent on the amino acid composition must be high.
3. It must be acceptable to the general public.
4. It must have the functionality, i.e. characteristics, which are found in common staple foods.
5. The economic viability of the SCP process is extremely complex and is yet to be demonstrated

BP Process – Candida on n-paraffin

Mechanically agitated fully baffled fermenters with turbine mixers. Reactor feed consists of paraffin, gaseous ammonia and other salts. Oxygen requirements per unit biomass produced by aerobic micro-organisms grown on n-hexadecane is 2.5 times higher than that required for growth on glucose and amounts to 2.2 kg O₂ per kg biomass.

Heat produced was 25000 kJ/kg biomass. Fermenters require substantial agitation. Because of the insoluble nature of alkanes, they exist in agitated fermenter as suspensions of alkane drops 1-100mm in diameter. Cell recovery is by centrifugation, producing 15% dry solids, evaporation to 25% dry solids and spray drying.

ICI Process – *Methylophilus methylotrophus* from methanol.

Pressure recycle fermentor. A combination of an airlift and loop reactor consisting of an airlift column, a down-flow tube with heat removal and a gas-release space. The nitrogen source is ammonia gas and pH is controlled between 6 and 7. Cell specific growth rate is approximately 0.5h⁻¹ and cell yield of 0.5 g/g. Methanol is oxidised via dehydrogenation to formaldehyde which can either be assimilated for conversion to cell mass or further oxidised to CO₂ with concomitant energy production. Cells are recovered by agglomeration followed by centrifugation, flash dried and ground.

Bel Fromageries process: *Kluyveromyces marxianus* from whey.

Whey which contains about 5% lactose, 0.8% protein and 0.2-0.6% lactic acid, is used as a substrate. Biomass production requires an aerobic fermentation whereas aeration is minimal for ethanol production. For feed grade biomass, the entire fermentation minerals and lactic acid may be recovered. For preparation of food grade material, cells are harvested by centrifugation, washed and dried. Cell yield is 0.45-0.55 g/g based on lactose consumed.

RHM Mycoprotein process: *Fusarium graminearum* from glucose.

Medium components include food grade glucose syrup, gaseous ammonia, salts, and biotin. Fermentation pH is controlled at 6 by gaseous ammonia addition, fed into the air inlet stream. Cell concentrations are 15-20g/L and a specific growth rate of up to 0.2h⁻¹ is achieved. Following cyclone separation and an RNA reduction step, cells are recovered by rotary vacuum filtration and formulated into a range of products.

Potential Substrates for SCP Production

Sulphite waste liquor

Candida utilis has been produced as a protein supplement by fermentation of sulphite waste liquor in Germany during both world wars. More recently a Finnish company developed a fungal SCP production process, the Pekilo Process, to grow *Paecilomyces varioti* using sulphite waste liquor.

Cellulose

Cellulose from natural sources and waste wood is an attractive starting material for SCP production because of its abundance. The association of cellulose with lignin in wood makes it somewhat intractable to microbial degradation. Thermal or chemical pretreatment, used in

combination with enzymatic hydrolysis, is usually required. Systems using cellulolytic organisms appear to have promise, but economic viability has yet to be achieved.

Whey

Whole milk whey or deproteinised whey is a carbohydrate source, which creates disposal problems. (High BOD) Problems associated with whey for SCP production are usually insufficient substrate, seasonal supply variations and its high water content (>90%) which makes transport prohibitively expensive. While most organisms do not grow on lactose as a carbon source, strains of the yeast *Kluyveromyces marxianus* readily grow on lactose.

Starch

The symba process was developed in Sweden to produce SCP from potato starch using two yeast strains. *Saccharomyces fibuligera* produces the enzyme necessary for starch degradation enabling co-growth of *Candida utilis*. This process is known as simultaneous saccharification and fermentation.

Glucose

Food grade glucose was the substrate chosen by RHM for production of fungal SCP using *Fusarium graminearum*. The strategy adopted was to take advantage of mycelial fibre content to produce a range of high added value products including meat analogues for human consumption.

Higher Alkanes

The original alkane SCP fermentation process, developed by BP in France used 10-20% wax contained in gas oil. Substrate costs were very low, however due to their crude nature, exhaustive processing was required to recover the yeast free of a gas-oil flavour taint. Other alkane based SCP processes were developed in Italy, Japan and Romania but many of them suffered from the problems of potential carcinogenic residues and most of the plants have never run on full capacity or have been closed.

Methane/Methanol

Methane was initially considered as a SCP raw material because, as a gas product, purification problems after fermentation would be minimal. Disadvantages associated with methane-based processes are related to: (a) the greater oxygen requirements necessary to fully oxidise methane compared with paraffins, (b) the low solubility of methane in water and (c) the requirement that the fermentation plant be flame proof as methane-oxygen mixtures are highly explosive. Methane is however easily converted to methanol which requires less oxygen, less fermenter cooling, is highly water soluble and has minimal explosion risks. ICI, which manufactures bulk methanol, chose this substrate for bacterial SCP production for animal feed using the trade name Pruteen. The company designed a non-mechanical "pressure cycle fermenter" which uses air for both agitation and aeration in the world's largest single aerobic fermenter of 3000m³ capacity. The process which produces 50-60,000 tonnes SCP per year, using the organism *Methylophilus methylotrophus*, was commissioned in 1979-1980, but has suffered from dramatic increases in methanol prices. The economic difficulties encountered by ICI with animal feed processes lead to a joint venture with RHM to produce *Fusarium* SCP in the ICI plant.

Choice of Microorganism

The key criteria used in selecting suitable strains for SCP production should consider the following:

The substrates to be used as carbon energy and nitrogen source and the need for nutrient supplementation.

High specific growth rates, productivity and yields one given substrate.

P^H and temperature tolerance.

Aeration requirements and foaming characteristics.

Growth morphology in the reactor.

Safety and acceptability – non pathogenic, absence of toxins.

Ease of recovery.

Protein, RNA and nutritional composition of the product.

Structural properties of the final product.

In general, fungi have the capacity to degrade a wider range of complex plant materials, particularly plant polysaccharides.

They can tolerate low pH which contributes to reducing fermenter infections.

Growth of fungi as short, highly branched filaments rather than in pellets is essential in order to optimize growth rate.

Bacteria, in general, have faster growth rates than fungi and grow at higher temperatures, thereby reducing fermenter cooling requirements.

Bacterial and yeast fermentations are easier to aerate. In contrast to fungi, which are easily recovered by filtration, bacteria and yeast require the use of sedimentation techniques and centrifugation. Bacteria, in general produce a more favourable protein composition than yeast or fungi. Protein content in bacterial can range from 60-65% whereas fungi selected for biomass production and yeast have protein contents in the range of 33-45%. However, associated with the higher bacterial protein levels is a much higher level of nutritionally undesirable RNA content of 15-25%. Microorganisms involved in SCP production must be safe and acceptable for use in food. Organisms should be stable genetically so that the strain with optimal biochemical and physiological characteristics may be maintained in the process through many hundreds of generations.

Microorganisms Raw Materials

Bacteria	Hydrogenomonas, Pseudomonas, etc.	Cellulomonas, Hydrocarbon wastes from petroleum industry.
Fungi		
Yeasts :	Candida spp., fragilis, Torula sp., Rhodotorula spp.	Whey, ethyl alcohol, starches, n-paraffins, sulphite waste, etc.
Moulds :	Aspergillus terreus, reesei, Penicillium spp.	Paper-pulp, starches, coffee wastes, straw, bassage, etc.
Algae	Scenedesmus acutues, acutues, Spirulina, Chlorella	In culture

Economics of SCP Production

The initial reasoning by companies such as BP and ICI to enter SCP production was to produce, at low cost, high value SCP from petroleum, for addition to animal feed. The intention was to replace imported protein additives such as soyabean meal. Factors which contributed to the failure of hydrocarbon SCP to make a major commercial impact included the 1973 dramatic oil price increases, which raised feedstock and energy costs and the lower price increases achieved by agricultural products such as soyabean. When one considers that crude oil prices increased by a factor of 6 in 1973, and that the cost of substrate for SCP processes represents 40-60% of total manufacturing costs, the negative impact on hydrocarbon based SCP processes may be understood.

Agricultural crops, the major competitor to SCP for animal feed, manifest a remarkable ability to respond to market forces and maintain price stability. By producing human protein supplements, the end value of the product can be appreciated, however the production process has to utilize more conventional and costly substrates for production (glucose). These products take advantage of the high fiber content in certain fungi to produce a product that is a good meat analogue. This product is also low in sodium and fat. The product Quorn is a fungal protein.

Advantages of Single-Cell Protein Manufacture

- (i) Microorganisms grow very vigorously and produce a high yield. It has been calculated that 100 lbs of yeast produces about 250 tons of protein within 24 hours. Algae grown in ponds produce 20 tons (dry weight) of protein per acre/year. The yield of protein is 10-15 times higher than soybeans and 20-50 times higher than corn.
- (ii) Industrial wastes or by-products are utilized as raw materials for microorganisms.
- (iii) The protein content in the cells of microorganisms is reported to be very high 60% protein in dried cells of *Pseudomonas* spp; 40-50% in yeast cells and 20-40% in algal cells have been calculated.
- (iv) Yeasts grown in this process possess high vitamin content.
- (v) All essential amino acids are contained by single-cell proteins.

Disadvantages of single-cell protein manufacture

- (i) Yeasts show lower growth rates, lower protein and lower methionine content.
- (ii) Moulds also have their limitations due to lower growth rates and lower protein content.
- (iii) Algae have cellulose in their cell walls which are not digestible. They also accumulate heavy metals which may prove harmful to living beings.
- (iv) Since the bacterial cells are small in size and have low density, their harvesting from the fermented medium becomes difficult and costly.

(v) Bacterial cells possess high nucleic acid content which may prove detrimental to human beings by increasing the uric acid level in blood. Additional steps to overcome this problem make the production costly.

Composition of Single Cell Proteins - Yeast single-cell protein (SCP) is a high-nutrient feed substitute. This study evaluates the dual applications of a novel recombinant *Pichia pastoris* SMD1168H (SMD) yeast, expressing a tilapia vitellogenin protein (rVtg), as an SCP diet for *Artemia* and the first-feeding fish larvae. Instar II *Artemia* fed rVtg, rVtg precultured in 5% fish oil (rVtg-FO), *Saccharomyces cerevisiae* (SC), or native SMD had greater lipid contents ($P < 0.05$) than the freshly hatched. Lipid deposition in the *Artemia* fed rVtg or rVtg-FO was greater ($P < 0.05$) than in those fed SMD or SC. Diet-induced accumulation of low levels of docosahexaenoic acid [22:6(n-3)] was detected only in *Artemia* fed the rVtg-based diets. Tilapia (*Oreochromis mossambicus*) larvae were fed solely yeast diets singly or in combination (d 3–22), or a staggered regimen of yeast (d 3–12) followed by unenriched or yeast-enriched *Artemia* (d 13–22). The larvae fed rVtg for 22 d increased in length and weight ($P < 0.05$), whereas those fed SC or SMD suffered growth suppression and high mortality. Such adverse consequences were ameliorated when 50% of SC was substituted with rVtg. The larvae prefed rVtg followed by a dietary switch to *Artemia* preenriched for 48 h with rVtg or rVtg-FO were greatest in length, had the highest weight gain, and lived the longest. Besides delivering rVtg protein, essential fatty acids and amino acids, rVtg may have probiotic effects in enhancing larval survival.

Single Cell Protein Production Product Safety and Quality

SCP has applications in animal feed, human food and as functional protein concentrates. Some bacterial SCP have amino acid profiles similar to animal/plant protein. Yeast, fungal and soya bean proteins tend to be deficient in methionine. Ingestion of RNA from non-conventional sources should be limited to 50g per day. Ingestion of purine compounds arising from RNA breakdown, leads to increased plasma levels of uric acid, which can cause gout and kidney stones. High content of nucleic acids causes no problems to animals since uric acid is converted to allantoin which is readily excreted in urine. Nucleic acid removal is not necessary from animal feeds but is from human foods. A temperature hold at 64C inactivates fungal proteases and allows RNA-ases to hydrolyse RNA with release of nucleotides from cell to culture broth. A 30 min stand at 64C reduces intracellular RNA levels in *Fusarium graminearum* from 80mg/g to 2mg/g.

Advantages of SCP

However, in many countries, use of microbial biomass as a supplement to diet has met with scepticism because of certain psychological barriers. But even in these countries, it will play a major role via SCP feeding to animals which will be then consumed by the humans. As compared with traditional methods of producing proteins for food or feed, large scale production of microbial biomass has the following advantages:

- (i) Micro organisms in general have a high rate of multiplication.
- (ii) Microbes have high protein content.
- (iii) They can utilize a large number of different carbon sources, some of which are waste

- iv) Strains with high yield and good composition can be selected or produced relatively easily.
- (v) Microbial biomass production is independent of seasonal and climatic variation.products.

Disadvantages of SCP

However, there are certain disadvantages also, of using microbial biomass as supplement to diet. Many micro organisms produce toxic substances and it has to be made sure that the biomass does not contain any such substance.

Sometimes the micro organisms when taken in, lead to indigestion and allergenic reactions. The high nucleic acid in many microbial biomass products is also undesirable

There are several limitations in the use of SCP for human consumption and they are due to

- (1) high nucleic acid contents of many microorganisms that would result in kidney stone formation or gout
- (2) poor digestibility, gastrointestinal problems and skin reactions and
- (3) the possible presence of toxic or carcinogenic compounds from residues of substrates.

The high capital costs and the need for sterility controls render SCP expensive in developing countries where food shortages are common. As animal feed, SCP grown on agricultural residues should certainly find a place in the future economy of developing nations. It is however noteworthy that Dabur in India is manufacturing and selling spirulina for human consumption.

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