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 proce 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 optimiseyield 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.

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.

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.

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 rhan that required for growth on glucose and amounts to 2.2 kg O_2 per kg biomass.

Heat produced was 25000 kJ/kg biomass. Fermenters require substantial agitation.

Because of the insoluble nature of alkanes, they exist in agiteated fermenter as suspensions of alkane drops 1-100μm 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 CO_2 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 contents, containing yeast, residual whey proteins, 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 insifficient 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 backterial 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 worlds largest single aerobic fermenter of 3000m3 capacity.

The process which produces 50-60,000 tonnes SCP per year, using the organism *Methylophilus methylotrophus, was comissioned 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 venutre 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 on a given substrate.

pH 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 optimise 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.

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 uncluded 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 utilise more conventional and costly substrates for production (glucose).

These products take advantage of the high fibre 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.