Microbial Biomass production:

In biomass production, the cells produced during the fermentation process are the products. Consequently, the fermentation is optimized for the production of a maximum concentration of microbial cells. Microbial biomass is broadly used for three purposes:

1-viable microbial cells are prepared as fermentation starter cultures and inoculum for food and beverage fermentations, waste treatment processes, silage production, agricultural inoculants, mineral leaching and as biopesticides.

2-as a source of protein for human food, because it is often odorless and tasteless and can therefore be formulated into a wide range of food items.

3- as animal fodder.

In a few instances the cells i.e. biomass of microbes, has industrial application as listed in table1. The main example is the production of baker's yeast and single cell proteins (SCP) which are in fact whole cells of *Spirullina* (an algae), *Saccharomyces* (a yeast) and *Lactobacillus* (a bacterium).

Table-1- Microbial biomass production

Product	Source	Use
Baker's yeast	*Saccharomyces cerevisae*	Production of bread, Single cell protein
Pruteen	*Methylophylus methylotrophus*	Single cell protein
Spirullina protein	*Spirulina maxima*	Single cell protein
Mushroom	*Agaricus bisporus*	Single cell protein
Bioleaching agent	*Thiobacillus ferrooxidans*	Solubiliztion of metals from ores
Bioinsecticide	*Bacillus thuriengensis*	Control of cotton eating insects

Baker's yeast:

In human diets, microbial protein constitutes a relatively minor portion of the total protein consumed. This primarily comes from edible macrofungi (mushrooms, truffles, etc.) and a small contribution in the form of yeast in bread. A major fermentation industry has developed to manufacture the vast quantity of baker's yeast required for making bread and associated bakery products.

Baker’s yeast belongs to *Saccharomyces cerevisiae*. There are many different strains in this group, all of them having somewhat different qualities. The yeast manufacturer therefore takes to find out strain most suitable to various local conditions.

As with all organisms, yeast needs energy source, carbon source, electron source, nitrogen, minerals, growth factors, water, and oxygen for growth and reproduction.

Improved strains are chosen for the following properties:

1- high glycolytic activity, a key feature being CO_2 generation rates or ‘gassing power’
2- rapid utilization of maltose, which is the main sugar of bread dough
3- Osmo tolerance, i.e. the ability to function in the presence of high levels of sugars and salt within dough
4- high growth rates.
5- Stability on storage
6- Ability to withstand drying
7- Low alcohol generation
8- Ability to disperse readily in water
9- Mild flavor
10- Ability to propagate readily

Baker’s yeast production:

- **Propagation of a starter culture**: which originates from a pure freeze-dried sample or agar-medium culture. Yeast cells are initially transferred to small liquid culture flasks, then on to larger intermediate vessels before being finally used to inoculate the large production fermenters of 50–350m^3 capacity. Overall, this may involve up to eight scale-up stages to produce the necessary final inoculum volume.

Medium for the production: Medium contains molasses as the carbon and energy source, which may be pretreated with acid to remove sulfides and heated to precipitate proteins. Molasses is often deficient in certain amino acids, and supplements of biotin and pantothenic acid are usually necessary. Further nitrogen sources (ammonium salts or urea) may be added, along with orthophosphate and other mineral ions, and the pH is adjusted to 4.0–4.4.

-Fermentation: The main objective of the process is to generate a high yield of biomass that exhibits an optimal balance of properties, including a high fermenting activity and good storage properties. **Aerobic fermentation** favors a **high biomass** yield, as approximately 50% of the available carbon can be potentially converted to biomass.

The maximum theoretical growth yield (Ys) is 0.54 g/g, whereas under anaerobic conditions this value reduced to around 0.12 g/g. Therefore, the aim is to maintain the cells under aerobic conditions. This is partially achieved by strong aeration of the fermentation broth, which is usually further increased as the fermentation progresses, via an aeration system.

In addition, the fermentation is operated as a fed-batch process. The nutrients are added at a specific rate, this feeding regime limits anaerobic metabolism and ethanol production, which would otherwise result in lower biomass yields. By maintaining a specific growth rate at 28–30°C, cell concentrations of up to 60 g/L can be achieved.

At the end of the growth phase, when all the nutrients are depleted, the aeration is continued for a further 30min to 'ripen' the yeast this period is called yeast ripening phase which encourages production of the storage carbohydrate trehalose, reduces protein and RNA synthesis, and stabilizes the cells to give a longer storage life.

-Separation and Filtration: Centrifugal separators, run at a minimum speed, are used to remove the yeast from the fermentation broth. Harvested cells are then washed several times with water, chilled to 2–4°C and finally the yeast cream is further concentrated by filtration on rotary vacuum filters or filter presses. Filtration yields a yeast cake of about 25–30% dry matter content.

Packaging: After filtration, the yeast cake is mixed with oils, emulsifiers, and a small amount of water, then compressed and extruded into blocks, or granulated for bulk distribution.

The Product:

- **Compressed yeast**

Compressed yeast is the traditional form of baker's yeast, which is available to wholesale bakers. It is dried to around 70–75% (w/w) moisture using vacuum filter dehydrators, the resulting fresh cream yeast that compressed in to blocks blended with suitable plasticizer (e.g. dextrin, pectin) and emulsifiers, to improve product appearance and aid the formation of blocks (extrusion, cutting), and then extruded in the form of rectangular blocks. Packaging is done in wax paper, and storage of packaged yeast under refrigeration. The compressed yeast has a storage life of 10 days at 5-8°C.

- **Dried yeast**

The yeast may be dried further to 5–10% (w/w) moisture to form dried yeast, which can then be stored for long periods without refrigeration. There are two forms of dried yeast.

1- **Active dried yeast (ADY)** needs to be rehydrated in warm water before use.
2- **Instant dried yeast (IDY)** does not require rehydration and can be mixed directly with flour in making dough.

Addition of a variety of agents to the yeast cake prior to drying can improve activity and stability of dried yeast. EX:Emulsifiers facilitate rehydration of IDY, and antioxidants increase the stability of ADY.

*Overall, the cycle of baker's yeast production, from the initial pure yeast sample to the yeast ready for sale, takes about 2 weeks.

Single cell protein:

Single cell protein (SCP) is not pure protein but refers to the whole cells of bacteria, yeasts, filamentous fungi or algae, and also contains carbohydrates, lipids, nucleic acids, mineral salts and vitamins. The protein content and quality are largely dependent on the specific microorganism utilized and the fermentation process. Fast-growing aerobic microorganisms are primarily used due to their high yields and high productivity (Table 2). A safety aspect that must be considered for all SCP products is nucleic acid content.

Many microorganisms have naturally high levels, because fermentation conditions favoring rapid growth rates and high protein content also promote elevated RNA levels. This can be problematic as the digestion of nucleic acids by humans and animals leads to the generation of purine compounds. Their further metabolism results in elevated plasma levels of uric acid, which may crystallize in the joints to give gout-like symptoms or forms kidney stones.

Table 2: Protein and nucleic acid content of microorganisms

Microbe	Protein percentage	Nucleic acid
Bacteria	50–85	10–16%
Yeast	45–55	5–12%
Filamentous fungi	30–55	3–10%
Algae	45–65	4–6%

Note: soya beans contain approximately 40% protein.

Microorganisms used in SCP production

Organisms to be used in SCP production should have the following properties:

(a) Absence of pathogenicity and toxicity.

(b) Protein quality and content: The amount of protein in the organisms should not only be high but should contain as much as possible of the amino acids required by man.

(c) Digestibility and organoleptic qualities of produced protein.

(d) Growth rate: It must grow rapidly in a cheap, easily available medium.

(e)It should be able to show tolerance towards pH and temperature.

(f) The microorganism must be easily available for harvesting with high protein yield.

A variety of bacteria, mold, yeast and algae are being employed in SCP production are listed in Table 3.

Table 3: Microorganisms used for SCP production

Bacteria	Algae	Yeast	Fungi
Brevibacterium spp.	*Chlorella pyrenoidosa*	*Candida utilis*	*Chaetomium celluloliticum*
Methylophilus methylitropous	*Chlorella sorokiana*	*Candida tropicalis*	*Fusarium graminearum*
Acromobacter delvaevate	*Chondruscrispus*	*Candida novellas*	*Aspergillus fumigates*
Bacillus megaterium	*Scenedus musacutus*	*Candida intermedia*	*Aspergillus niger*
Lactobacillus spp.	*Porphyrium sp*	*Saccharomyces cerevisiae*	*Rhizopuschinensis*
Cellulomonas spp.	*Spirulina maxima*		*Tricodermaviridae*

Microorganisms and their properties

(a) Bacteria:

They are generally high in protein and possess faster growth rate. The disadvantages include the small size of bacterial cells and low density which affects harvesting of biomass from fermented medium, which ultimately increases the cost of operation. The nucleic acid content is quite high in bacteria which have the tendency to increase uric acid level in human blood upon consumption.

(b) Yeast:

The larger size of yeast facilitates easier harvesting of the product. The lower nucleic acid content, high lysine content and ability to grow in acidic pH are the added benefits of yeast. Despite this, yeast has lower growth rate, low protein content and low methionine content, when compared to bacteria.

(c) Fungi:

Filamentous fungi are easy to harvest, but they have low productivity, because of their lower growth rate and low protein content.

(d) Algae:

The cellulosic cell walls in it that are hard to digest by human beings. There are also chances of concentrations of heavy metals in the product, when algae are used in the medium.

Single cell protein production process:

The SCP production processes essentially contain the same basic stages irrespective of the carbon substrate or microorganism used.

1- Medium preparation: The main carbon source may require physical or chemical pretreatment prior to use. Polymeric substrates are often hydrolyzed before being incorporated with sources of nitrogen, phosphorus and other essential nutrients.

2- Fermentation: The fermentation may be aseptic or run as a ‘clean’ operation depending upon the particular objectives. Continuous fermentations are generally used, which are operated at close to the organism’s maximum growth rate, to fully exploit the superior productivity of continuous culture.

3- Separation and downstream processing: The cells are separated from the spent medium by filtration or centrifugation and may be processed in order to reduce the level of nucleic acids. This often involves a thermal shock to inactivate cellular proteases. RNase activity is retained and degrades RNA to nucleotides that diffuse out of the cells. Depending upon the growth medium used, further purification may be required, such as a solvent wash, prior to pasteurization, dehydration and packaging.