Single Cell Protein

single cell protein is a protein extracted from cultured algae, yeasts, or bacteria and used as a substitute for protein-rich foods, especially in animal feeds or as dietary supplements. Many types of animal feeds contain single cell proteins.

60-80% dry cell weight; contains nucleic acids, fats, CHO, vitamins and minerals
Rich in essential amino acids (Lys-Met)

Microbes can be used to ferment some of the vast amounts of waste materials, such as straws; wood and wood processing wastes; food, cannery and food processing wastes; and residues from alcohol production or from human and animal excreta.

- 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.

- 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.

Advantages of using Microorganisms

1. MO grow at very fast rate under optimal conditions
2. Quality and quantity is better than higher plants and animals
3. Wide range of raw materials can be used
4. Culture and fermentation conditions are simple
5. MO can be genetically manipulated

Limitations of using SCP

1. Nucleic acid content is very high (40% algae; 10-15% bacteria and 5-10% yeast)
2. Presence of carcinogenic and toxic substances
3. Contamination of pathogenic MO
4. Indigestion and allergic reactions
5. Production of foodgrade SCP is expensive

Some SCPs…

Microbes employed include

Yeasts
Saccharomyces cerevisiae,
Pichia pastoris,
Candida utilis=Torulopsis and
Geotrichum candidum (=Oidium lactis)),

other fungi
Aspergillus oryzae,
Fusarium venenatum,
Sclerotium rolfsii,
Polyporus and
Trichoderma),

Bacteria
Rhodopseudomonas capsulata

Algae
Chlorella and
Spirulina

Typical yields of 43 to 56%, with protein contents of 44% to 60%.

Microorganism	Substrate
Bacteria	
Aeromonas hydrophylla	Lactose
Acromobacter delvacvate	n-Alkanes
Acinetobacter calcoacenticus	Ethanol
Bacillus megaterium	Non-protein nitrogenous compounds
Bacillus subtilis, *Cellulomonas* sp., *Flavobacterium* sp., *Thermomonospora fusca*	Cellulose, Hemicellulose
Lactobacillus sp.	Glucose, Amylose, Maltose
Methylomonas methylotrophus, M. clara	Methanol
Pseudomonas fluorescens	Uric acid and other non-protein nitrogenous compounds
Rhodopseudomonas capsulata	Glucose
Fungi	
Aspergillus fumigatus	Maltose, Glucose
Aspergillus niger, A. oryzae, *Cephalosporium eichhorniae*, *Chaetomium cellulolyticum*	Cellulose, Hemicellulose
Penecillium cyclopium	Glucose, Lactose, Galactose
Rhizopus chinensis	Glucose, Maltose
Scytalidium aciduphlium, *Thricoderma viridae*, *Thricoderma alba*	Cellulose, pentose
Yeast	
Amoco torula	Ethanol
Candida tropicalis	Maltose, Glucose
Candida utilis	Glucose
Candida novellas	n-alkanes
Candida intermedia	Lactose
Saccharomyces cereviciae	Lactose, pentose, maltose
Algae	
Chlorella pyrenoidosa, *Chlorella sorokiana*, *Chondrus crispus*, *Scenedesmus* sp., *Spirulina* sp., *Porphyrium* sp.	Carbone dioxide through photosinthesis

SCP can be produced from high energy sources:

Alkanes, methane, ethanol, methanol, gas oil

Generally bacteria and yeasts are employed

Pekilo: a fungal protein rich product

Paecilomyces variotii is used for production of Pekilo

This protein was produced by fermentation of wastes such as molassess, whey, sulfite liquor and agricultural wastes

Quorn: mycoprotein for humans

Produced by *Fusarium graminearum*; It is dried and artificially flavoured and marketed in pieces that resemble beef, pork and chicken. Rich in essential nutrients and good content of dietary fibre.

Single cell protein has the potential to be developed into a very large source of supplemental protein that could be used in livestock feeding.

Methods available for concentrating include, filtration, precipitation, coagulation, centrifugation, and the use of semi-permeable membranes. These de-watering methods require equipment that is quite expensive and would not be suitable for most small-scale operations. Removal of the amount of water necessary to stabilize the material for storage, in most instances, is not currently economical.

Single cell protein must be dried to about 10 % moisture, or condensed and acidified to prevent spoilage from occurring, or fed shortly after being produced.

A wide range of substrates can be used to grow microbial proteins

whey, orange peel residue, sweet orange residue, sugarcane bagasse, paper mill waste, rice husks, wheat straw residue, cassava waste, sugar beet pulp, coconut waste, yam waste, banana pulp, mango waste, grape waste, sweet potato

Single cell protein was a suitable supplemental protein source for lactating dairy goats.

Milk production and milk production efficiency was increased when single cell protein replaced groundnut meal in lactating goat diets

SCP from sewage
wood
wastes
High energy sources like methanol, alkanes, methane, ethanol

Why do we need need alternative sources of food?

About 50 years ago (1934-1938) the less developed areas of the world, Asia, Africa and South America, were the main exporters of grain to the developed world.

Since 1948 the food flow has reversed, from the developed world to the less developed, mainly due to the rate of growth of the world's population which was much higher in the less developed countries.

Based on present trends United Nations (UN) population experts project that there will be **8 billion people living on this planet by 2015 and 10.5 billion by the year 2110.**

This means that during the **35-year period (1980-2015) we must produce as much food as we have since the dawn of agriculture about 12000 years ago**.

Reality

Death from starvation, malnutrition and related diseases is a reality in many countries today.

The World Health Organisation (WHO) estimates that 12,000,000 people die of hunger and starvation related diseases every year. **Half are children under the age of 5.**

Microorganisms

Bacteria

Methylophilus methylotrophus
Pseudomonas sp.
Brevibacterium sp.

Yeasts

Lactobacillus bulgaricus
Candida lipolytica
Bakers yeast
Kluyveromyces fragilis

Fungi

Trichoderma viridae
Aspergillus niger

Actinomycetes

Nocardia sp
Thermomonospora fusca

Mushrooms

Agaricus
Morchella
Vovariella

Properties of SCP

One of the main advantages of SCP compared to other types of protein is the small doubling time of cells (td) as shown below.

Mass doubling time (S)

Organism	Mass Doubling
Bacteria and yeast	10-120 min
Mold and algae	2-6 h
Grass and some plants	1-2 wk
Chickens	2-4 wk
Pigs	4-6 wk
Cattle	1-2 mo
People	0.2-0.5 yr

1

Due to this property, the productivity of protein production form micro-organisms is greater than that of traditional proteins

2

Efficiency of protein production of several protein sources in 24 hours

Organism (1,000 kg)	Amount of protein
Beef cattle	1.0 kg
Soybeans	10.0 kg
Yeast	100.0 tn
Bacteria	100x10,000,000 tn

It is assumed that the growth occurs without any restriction.

Other advantages of SCP over conventional protein sources are:

it is independent of land and climate;
it works on a continuous basis;
it can be genetically controlled;
it causes less pollution.

There are five factors that impair the usefulness of SCP:

1. non digestible cell wall (mainly algae);
2. high nucleic acid content;
3. unacceptable coloration (mainly with algae);
4. disagreeable flavour (part in algae and yeasts);
5. cells should be killed before consumption.

Thus SCP is treated with various methods in order to:

- kill the cells;
- improve the digestibility;
- reduce the nucleic acid content

Nutritional Value of SCP

Average composition of the main groups of micro-organisms (% dry weight)

	Fungi	Algae	Yeasts	Bacteria
Protein	30-45	40-60	45-55	50-65
Fat	2-8	7-20	2-6	1.5-3.0
Ash	9-14	8-10	5-9.5	3-7
Nucleic acids	7-10	3-8	6-12	8-12

Bacterial protein is similar to fish protein, yeast's protein resembles soya and the fungi protein is somewhat lower than the yeast's. Of course microbiological proteins are deficient in the sulphur amino acids cysteine and methionine and require supplementation, while they exhibit better levels of lysine

Essential amino acid content of the cell protein in comparison with several reference proteins (grams of amino acid per 100 grams of protein)

Amino acid	Cellulamonas	Saccharomyces cerevisiae	Spirulina maxima	Penicillium notatum	B P (SCP)	Wheat	Egg	Cow milk
Lysine	7.6	7.7	4.6	3.9	7.0	2.8	6.3	7.8
Threonine	5.4	4.8	4.6	–	4.9	2.9	5.0	4.6
Methionine	2.0	1.7	1.4	1	1.8	1.5	3.2	2.4
Cysteine	–	–	0.4	–	–	2.5	2.4	–
Tryptophane	–	1.0	1.4	1.25	–	1.1	1.6	–
Isoleucine	5.3	4.6	6.0	3.2	4.5	3.3	6.8	6.4
Leucine	7.3	7.0	8.0	5.5	7.0	6.7	9.0	9.9
Valine	7.1	5.3	6.5	3.9	5.4	4.4	7.4	6.9
Phenylalanine	4.6	4.1	5.0	2.8	4.4	4.5	6.3	4.9
Histidine	7.8	2.7	–	–	2.0	–	–	–
Arginine	6.4	2.4	–	–	4.8	–	–	–

The vitamins of micro-organisms are primarily of the B type, B12 occurs mostly in bacteria, while vitamin A is usually found in algae. Table shows the vitamin content of various food MO; Vitamin content of various food micro-organisms (mg/100 g dry weight)

Vitamin	Morchella Hortensis	Candida Utilis	Saccharomyces Cereviviae	Methylomonas Methanica
Thiamine	0.52	0.53	5-36	1.81
Riboflavin	1.31	4.50	3.6-4.2	4.82
Niacin	12.4	41.73	80-100	15-9
Pyridoxine	2.62	3.34	2.5-10	14.3
Pantothenic acid	12.6	3.72	10	2.42
Choline	4.61	-	-	968.0
Folic acid	1.09	2.15	1.5-8.0	-
Inositol	1.78	-	-	-
Biotin	0.015	0.23	0.5-1.8	-
Vitamin B_{12}	0	0	0	0.96
P-aminobenzoic acid	-	1.7	0.9-10	-

nutritional parameters which evaluate the quality of a given SCP are:

- the digestibility (D)
- the biological value (BV)
- the protein efficiency ratio (PER)
- the net protein utilisation (NPU)

The Problem of Nucleic Acids

About 70-80% of the total cell nitrogen is represented by amino acids while the rest occurs in nucleic acids.

This concentration of nucleic acids is higher than other conventional proteins and is characteristic of all fast growing organisms.

The problem which occurs from the consumption of proteins with high concentration of nucleic acids (78-25 g/100 g protein dry weight) is the high level of uric acid in the blood, sometimes resulting in the disease gout.

Uric acid is a product of purine metabolism. Most mammals, reptiles and molluscs possess the enzyme uricase, and the end product of purine metabolism is allantoin.

Man, birds and some reptiles lack the enzyme uricase and the end product of purine degradation is uric acid.

The **removal or reduction of nucleic acid content** of various SCP's is achieved with one of the following treatments:

chemical treatment with NaOH;
treatment of cells with 10% NaCl;
thermal shock.

These methods aim to reduce the RNA content from about 7% to 1% which is considered within acceptable levels.

SCP from n-Alkanes

In the late 1950's, British Petroleum (BP) became interested in the growth of a micro-organism in C_{12}-C_{20} alkanes. This constitutes the wax fraction of gas oils for treating. Some crude oils contain up to 15% in wax, and these waxes must be removed since they make oil more viscous, precipitate out at low temperatures, block tubes etc.

BP uses two yeasts, *Candidor lipolytica* and *C. tropicals* and built a 16,000 tons/year plant in Cap Lavera, France, and a 4,000 tons/year plant in England. The product produced was called **"TOPRINA"**. In the UK the product "TOPRINA G" was a purer product while the one in France was not separated from alkanes.

Both processes employed NH_3 as N-source and Mg ions to increase yields. No other carbon source was used.

For 12 years TOPRINA was tested for toxicity and carcinogenecity and was marketed as a replacement for fish meal in high protein feeds and as a replacement for skimmed milk powder in milk replacers.

There were no signs at all for toxicity or carcinogenicity. In spite of this, people were concerned that aromatic hydrocarbons may be carried over to SCP. The main opposition came from Japan, where environmental groups and university professors condemned SCP as dangerous, and the matter became political.

In 1972 a specialised committee decided that SCP was only for animal feeding but later, Japan was the first country to ban petrochemical protein.
The Italian government ordered further studies which showed that there was no hazard or carcinogenesis due to SCP.

Pigs fed on 30% TOPRINA in their diets showed less n-paraffins in their fat tissue than those fed on pasture. Based on this evidence the Italian government agreed to the use of TOPRINA in limited amounts and only for export.
In 1977 Italy stopped the SCP production for alkanes altogether due to the increase in oil prices. The price of soya was more competitive. Now there is no factory which produces any petrochemical protein.

SCP from Methane

Methane is cheap, abundant and without the toxicity problems of alkanes. It is a constituent of North Sea Gas and is also produced during anaerobic digestion.

Methane contains the most highly reduced form of carbon and consequently gives high cell yields relative to the amount of gas consumed.

The general *Methylomonas* and *Methylococcus* have been recognised as utilising methane as a carbon source. The species which has been extensively studied is *Methylomonas methanica.*

Nitrates or ammonium salts can serve as N-source.

Perhaps the most important work in this field was carried out by Shell in England. The process involves methane oxidation by stable mixed cultures. These were
1.a methane utilising G(-) rod;
2.a Hyphomicrobium;
3.two g(-) rods; *Acinetobacter and Flavobacterium*
This mixed culture was one of the best examples of symbiosis.

The process began in 1970 in a 300 e pilot plant at Sittingbourne, UK.

In spring 1976, Shell stopped commercialisation and its development plans were indefinitely postponed.

This decision was based on 3 factors:

1. the low price of soybeans & maize;
2. the potential of many countries for expanding existing protein sources;
3. the difficulty in applying Shell's sophisticated process in underdeveloped countries.

Shell

ICI: Imperial Chemical Industries

SCP from Methanol

The technology of SCP from methanol has been well studied and the most advanced process belongs to ICI.

The fermentation was carried out in a big airlift fermentor with the bacterium. *Methylophilus methylotropha*.

This organism was selected among other methanol utilisers after screening tests for pathogenicity and toxicity. As a nitrogen source ammonia was used. The product was named **"PRUTEEN".**

Pruteen contained 72% crude protein and was marketed for feed as a source of energy, vitamins and minerals as well as a highly balanced protein source. The methionine and lysine content of Pruteen compared very favourably with white fish meal.

ICI has commissioned a 60,000 tn/year plant utilising the single largest fermentor in the world (2 x 10,000,000 l).

Unfortunately Pruteen now cannot compete with soya and fish meal. ICI hopes to be able to sell their technology, because they have given up the idea of making money out of Pruteen. So today Pruteen although a major engineering success is not economical to run.

SCP from Ethanol

Ethanol although expensive as a substrate has been used for SCP.

The process comes from the Amoco Company in the US utilising a food grade yeast: "Torula". The product is sold by the name **"TORUTEIN"** and government clearances have been obtained to market Torutein in Canada and Sweden.

The yeast is about 52% protein and due to its relatively low Methionine level has a PER of about 1.7. The PER of wheat from 1.1 to 2.0.

Torutein is being marketed as a flavour enhancer of high nutritional value, and a replacement for meat, milk and egg protein.

However it is not very successful in the United States since soya which is plentiful and cheap can serve as an alternative or substitute to meat and egg diets.

Mycoprotein

This is a development of Ranks Hovis McDougall and is the only mycoprotein (except edible mushrooms) that has been cleared for human consumption.

It uses a *Fusarium graminearum* growing in molasses, or glucose.

The medium contains NH_3 for nitrogen source and pH control.

The product is heat treated for RNA reduction.
The mycelium is separated by vacuum filtration, and can be technologically treated to match food texture.

In the UK it is marketed as pies and is considered a success since having less fat than meat, it can be sold at a premium price.

SCP from Lignocellulose

Mushrooms

The lignocellulosic wastes, mainly from agriculture, constitute the most abundant substrate for SCP which is also renewable. The world annual production of straw for example reaches 600 million tons every year. In Greece the straw from wheat and rye, the two most important cereals, is an estimated 1.5 million tons per year.

For the utilisation of lignocellulose, a pre-treatment is usually necessary. Many pre-treatment methods have been reported which vary from alkali or acid treatment, steam explotion or even x-ray radiation.

To the present time the only economical utilisation of lignocellulosic wastes is in mushroom production.
Besides our well know cultivated mushroom *Agaricus bisporus* there are many important ones which contain lignocellulolytic enzymes and are cultivated for food mainly in Asia and Africa.

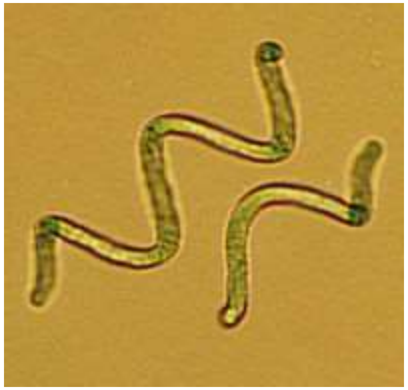
Some are of great economic significance and are cultivated on an industrial scale. Examples of important ones include the following species: *Volvariella* sp., *Lentinus edodes* and *Pleurotus* sp

Spirulina

Common name for human and animal food supplements produced primarily from two species of cyanobacteria (also known as blue-green algae): *Arthrospira platensis*, and *Arthrospira maxima*.Use only CO_2 and sunlight

Used as a human dietary supplement as well as a whole food and is available in tablet, flake, and powder form.

It is also used as a feed supplement in the aquaculture, aquarium, and poultry industries



Nutrients

Protein

Spirulina contains an unusually high amount of protein, between 55% and 77% by dry weight, depending upon the source. It is a complete protein, containing all **essential amino acids**, though with reduced amounts of methionine, cysteine, and lysine when compared to the proteins of meat, eggs, and milk. It is, however, superior to typical plant protein, such as that from legumes

Essential fatty acids

Spirulina is rich in gamma-linolenic acid (GLA), and also provides alpha-linolenic acid (ALA), linoleic acid (LA), stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA)

Vitamins

Spirulina contains vitamin B1 (thiamine), B2 (riboflavin), B3 (nicotinamide), B6 (pyridoxine), B9 (folic acid), vitamin C, vitamin D, and vitamin E. A one gram tablet could provide more than three times the recommended daily intake of B12.

Minerals

Spirulina is a rich source of potassium, and also contains calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, and zinc.

Photosynthetic pigments

Spirulina contains many pigments including chlorophyll-a, xanthophyll, beta-carotene,zeaxanthin, canthaxanthin, plus the phycobiliproteins c-phycocyanin and allophycocyanin.

Chlorella ***Senedesmus***

single-celled green algae without flagella

Contains the green photosynthetic pigments chlorophyll-a and -b in its chloroplast.

Through photosynthesis it multiplies rapidly requiring only carbon dioxide, water, sunlight, and a small amount of minerals to reproduce

Potential source of food and energy because its photosynthetic efficiency can, in theory, reach 8%, comparable with other highly efficient crops such as sugar cane.

It is also an attractive food source because it is high in protein and other essential nutrients; when dried, it is about 45% protein, 20% fat, 20% carbohydrate, 5% fiber, and 10% minerals and vitamins. However, because it is a single-celled algae, harvest posed practical difficulties for its large-scale use as a food source. Mass-production methods are now being used to cultivate it in large artificial circular ponds

SCP's Evaluation and Future Prospects

The development of SCP was really the beginning of biotechnology.

Prior to this the industrial fermentation was mainly focused on antibiotics and other products which did not have to compete.

This was not the case with SCP which had to compete with similar products in the market.

The development was brought up by the oil companies rather than the food companies, because they could take the risk of a highly costly product out with no real expected profit. They also had all the high technology required.
The efforts tried so far by adding dry SCP as a supplement to diets in order to solve the problems of the hungry in the Third World Countries, certainly have not given the expected results.

Every new food which appears in the market should have not only high nutritive quality, but also satisfactory organoleptic (*Organoleptic* refers to any sensory properties of a product, involving taste, colour, odour and feel) supplementary element.

Today in most countries where market forces operate SCP cannot compete with soya, alfalfa or fish meal.

Mushroom production from lignocellulosics seems to be one economical and promising use for SCP.

For future success of SCP, first, food technology problems have to be solved in order to make it similar to familiar foods and second, the production should compare favourably with other protein sources.

Mushrooms

Agaricus
Auricularia → Basidiomycetes

Morchella
Tuber → Ascomycetes

- 4000 species are known
- 200 are edible and dozens are cultivable
- Microbial culture is macroscopic and used as food directly
- Fastest growing biotech industries
- Expected for production of enzymes, pharmaceutical compds like antitumour agents and antibiotics

Poisonous Mushrooms

- Unpleasant taste and odour
- Produce toxic alkaloids or substances like Phalline and muscarine
- Eg. *Amanita phalloides*
- *A. muscaria, A. viraosa, Boletus*

A. Phalloides

A. muscaria

Boletus

Poisonous Mushrooms

- Many mushroom species produce secondary metabolites that can be toxic, mind-altering, antibiotic, antiviral, or bioluminescent. Although there are only a small number of deadly species, several others can cause particularly severe and unpleasant symptoms. (emetic)
- Psychedelic mushrooms : **Psilocybin mushrooms** possess psychedelic properties. Commonly known as "magic mushrooms" or "shrooms," they are openly available in smart shops in many parts of the world, or on the black market in those countries that have outlawed their sale. Psilocybin mushrooms have been reported as facilitating profound and life-changing insights often described as mystical experiences. *A.* muscaria also is psychoactive (ibotenic acid and muscimol)
- Many toxic or psychoactive mushrroms have been used for treat ment of psychiatric disorders like (OCD) and others like migranes but have side effects.

Psychedelic mushrooms : **Psilocybin mushrooms: magic mushrooms**

Medicinal Mushrooms

Medicinal mushrooms are mushrooms or extracts from mushrooms that are used or studied as possible treatments for diseases.

Some mushroom materials, including polysaccharides, glycoproteins and proteoglycans, modulate immune system responses and inhibit tumor growth.

Some medicinal mushroom isolates that have been identified also show cardiovascular, antiviral, antibacterial, antiparasitic, anti-inflammatory, and antidiabetic properties.

Currently, several extracts have widespread use in Japan, Korea and China, as adjuncts to radiation treatments and chemotherapy.

Pigments or chromophores are used in dyeing for packaging

Nutrition in Mushrooms

- Known as 'vegetable meat' for vegetarians
- 80-90% water (temp and humidity)

- Rich sources of protein (35-45% dry weight)
- All are not easy to digest
- Contain fats and free fatty acids (7-10%), CHO (5-15%) and minerals
- Heavy metals can also be found (Cd, Cr)

- Delicious recipes (mushroom soup, paper, paneer, pulao, egg)

- Mushrooms are a low-calorie food usually eaten raw or cooked to provide garnish to a meal.

- Raw dietary mushrooms are a good source of B vitamins, such as riboflavin, niacin and pantothenic acid, and the essential minerals, selenium, copper and potassium. Fat, carbohydrate and calorie content are low, with absence of vitamin C and sodium

- When exposed to ultraviolet light, natural ergosterols in mushrooms produce vitamin D2, a process now exploited for the functional food retail market.

- Disadv: have tendency for accumulating heavy metals and radioactivity (Chernobyl disaster)

Advantages of edible mushrooms

- Can be grown using waste substrates (cheap, industrial and wood wastes)

- High nutritive value being rich in proteins, minerals and vitamins
- There is high demand because of different ways they can be cooked
- Low CHO content good for diabetics

Production of Mushrooms

- Fermentation process
- Solid state fermentation
- Straw, saw dust, compost, wooden logs, horse dung, pig dung
- Low technology utilization in sophisticated modern biotechnology

- Most common are:
    - *Agaricus bisporus* (button mushrooms)
    - *Lentinula edodes* (2nd most cultivated) **Shiitake**

Paddy straw mushrooms: *Volvariella volvacea*

Pleurotus: Oyster mushrooms

Morchella esculenta : Morel

Most expensive and prized mushroom

Honeycomb like structure

Abundant after forest fires

Formulation and preparation of compost

↓ sterilization

Compost spread in trays

↓ Spawning

Spawn running

↓ ideal culture conditions
(temp (15oC), pH (7),O2, humidity (70-80%)

Stock culture

↓

Spawn preparation

→ (into spawn running stage)

Mushrooms

7-10 days
3-4 crops

↓

Harvesting

↓

Marketing

Life of mushrooms
8-12h unless stored at low temp (2-5oC)
Or consumed, stored, canned, lyophilized