PPT Bank by DAW

- 1. Citric acid production
- 2. Sterilization
- 3. SCP
- 4. L-Asparaginase
- 5. GMP

Sterilization

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 Sterilization (Latin *sterilis* meaning barren) is the complete removal or inhibition of all live forms (living cell, spores, virus, etc.) from any object or place. Petri plates, flasks, beakers, pipettes, etc.

Sterilization



- Dry Heat sterilization (Hot Air Oven)
- Moist Heat sterilization (Autoclave)
- Filter Sterilization
- Flame Sterilization
- Surface Sterilization(Using Chemicals)

Methods For Sterilization



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Metal instruments, glassware, aluminum foil, etc ., can be sterilized by exposure to hot dry air (130°-170°C) for 2-4 hr in a hot-air oven.
All items should be sealed before sterilization but not in paper, as it decomposes at 170°C.

Dry Heat sterilization(Hot Air Oven)



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- Autoclaving is a method of sterilizing with water vapor under pressure.
- Nearly all microbes are killed by exposure to the super-heated steam of an autoclave.
- Cotton plugs, gauze, lab ware, plastic caps, glassware, filters, pipettes, water, and nutrient media can all be sterilized by autoclaving, but autoclaving is not advisable for metal instruments due to the rust it may cause.

Moist Heat sterilization (Autoclave)



 sterilization as a process that eliminates (removes) or kills (deactivates) all forms of life and other biological agents.

Up until now all the sterilization methods we covered deactivate or kill bacteria and viruses.
But this method stops the bacteria's ability to reproduce.

Filter Sterilization





• Membrane filters are thin filters that are made of cellulose. They can be used for sterilization during injection by placing the membrane between the syringe and the needle.

• Seitz filters are usually made of asbestos. They are pad-like and thicker than membrane filters.

• Sintered glass filters are an alternative type of filter that are made of glass and hence do not absorb liquids during filtration.

 Candle filters are made of clay-like mud. This special mud has tiny pores made by algae. The microbes get stuck during their travel through the pores.



 Instruments can be dipped in ethyl alcohol and flamed to burn off all bacteria and fungi.

 Alcohol is flammable and if spilled near a flame will cause an instant flash fire.

Flame Sterilization





Surface sterilization of explant is a process which involves the immersion of explants into appropriate concentration of chemical sterilants or disinfectants for a specified time resulting in the establishment of a contamination-free culture.

Surface Sterilization



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10% v/v solution of liquid detergent (Teepol) for 10-15 min.

70% ethyl alcohol for 1 min. in front of laminar air flow.

Treatment with 0.1% HgCl2 (W/V) or 5-10% sodium hypochlorite.



Thank You....



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Over view

- Citric acid is a usually occuring acid found primarily in Several Varieties of fruits and vegetables with citrus fruits such as lemons and limes containing the highest amounts of citric acid.
- This Organic acid has many uses, including as a food additive /Preservative, ingredient in Cosmetic products and as a powerful cleaving agent.



Introduction:

Citric acid (2-hydroxy-1,2,3- propane tri carboxylic acid) is the most important commercial product, which is found in almost all plant & animal tissues.

Citric acid is the most important organic acid produced in tonnage and is extensively used in food and pharmaceutical industries. Citric acid is a weak organic acid found in citrus fruits(lemon). It is good ,natural preservative and is also used to add an acidic taste to food and soft drinks.

More than million tonnes are produced every year by fermentation.







HISTORY:

The discovery of citric acid has been credited to the 8th century Muslim alchemist Jabir Ibn Hayyan (Geber).

Citric acid was first isolated in 1784 by the Swedish chemist carl Wilhelm Scheele, who crystallize it from lemon juice.

Industrial scale citric acid production began in 1890 based on the Italian citrus fruit industry.

In 1893, C. Wehmer discovered penicillin mold could produce citric acid from sugar. However, microbial production of citric acid did not become industrially important until world war I disrupted Italian citrus exports.









Micro-organisms used for citric acid pro

Large number of micro-organisms including bacteria, fungi and yeasts have been employed to produce citric acid. The main advantages of using this micro-organisms are: a) Its easy of handling b) Its ability to ferment a variety of cheap raw materials

c) High yields



Micro organisms:

Fungi:

Aspergillus nagger

- A. aculeatus
- A. awamori
- A. carbonarius
- A. wentii
- A. foetidus

Penicillium janthinelum

Bacteria:

Bacillus licheniformis Arthrobacter paraffinens Corynebacterium species

Yeasts: Saccahromicopsis lipolytica Candida tropicalis C. oleophila C. guilliermondii C. parapsilosis C. citroformans

Hansenula anamosa





Citric acid production:

- Fermentation is the most economical and widely used ay for synthesis citric acid production.
- The industrial citric acid production can be carried in three different ways:
 - surface fermentation
 - submerged fermentation
 - solid state fermentation





Surface Fermentation:

- Surface fermentation using Aspergillus niger may be done on rice bran as is the case in Japan, or in liquid solution in flat aluminium or stainless steel pans.
- Special strains of Aspergillus niger which can produce citric acid despite the high content of trace metals in rice bran are used.



SUBMERGED FERMENTATION:

- In this case, the strains are inoculated of about 15cm depth in fermentation tank.
- The culture is enhanced by giving aeration using air bubbles.
- And its allowed to grow for about 5 to 14 days at 27 to 33 degree Celsius.
- The citric acid produced in the fermentation tank and it is purified.



Solid state fermentation:

- It is simplest method for citric acid production.
- Solid state fermentation is also known as koji process, was first developed in Japan.
- Citric acid production reached a maximum(88g/kg dry matter)when fermentation as carried out with cassava having initial moisture of 62% at 26degree Celsius for 120 hours.



Separation:

- The biomass is separated by filtration.
 - The liquid is transferred to recovery process
- Separation of citric acid from the liquid precipitation.
- Calcium hydroxide is added to obtain calcium citrate.





Separation process:





PURIFICATION:

- Purification is a simple form of getting a pure citric acid followed by two simple techniques.
- Precipitation
- Filtration

React citric acid with calcium carbonate Purified citric Acid Filter Purified citric Acid Filter precipitate Filter precipitate Filter precipitate









Factors affecting citric acid production:

- Nitrogen source
- ▶ pH
- Aeration
- Trace elements
- Temperature





Industrial production of citric acid:

99% of world production \implies microbial processes surface or submerged culture.

70% of total production of 1.5 million tons per year is used in food and beverage industry as on acidifier or antioxidant to preserve or enhance the flavors and aromas of fruit juices, ice cream and marmalades.

20% used \implies pharmaceutical industry as anti oxidant to preserve vitamins, effervescent, pH corrector, blood preservative, or in the form of iron citrate.



Tablets, ointments and cosmetic preparations

- Chemical industry remaining 10% softening and treatment of textile.
- Also used in the detergent industry as a Phosphate substitute, because of less entropic effect hardening of cement





Applications/uses of citric acid:

Food & drink:

Preservative and flavoring agent Emulsifying agent in ice-cream. Household cleaner:

Kitchen Bathroom sprays.

Cosmetics:

Shampoos Body wash WASH CLEANERS:

> Nail polish Hand soap and other cosmetic products




To cure kidney disorders:

Sodium citrate, acetic acid is used to prevent kidney stones.

Side effects:

Taking excess of citric acetate in combination with sodium citrate may lead to kidney failure. Taking citric acid with empty stomach may lead to stomach or intestinal side-effects. It may also lead to muscle twisting or cramps. It can also cause weight gain, swelling, fast heart rate, slow or rapid.



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Uses of Citric acid

Largest producer in the world:

country	Fruit crop	
USA	Grape fruit, pummelo	
China	Mandarin	
Brazil	Sweet orange	
India	Acid lime	
Italy	Lemon	



Good Manufacturing Practice (GMP)

"A GMP is a system for ensuring that products are consistently produced and controlled according to quality standards. It is designed to minimize the risks involved in any pharmaceutical production that cannot be eliminated through testing the final product".

✤GMP covers all aspects of production from the starting materials, premises and equipment to the training and personal hygiene of staff. Detailed, written procedures are essential for each process that could affect the quality of the finished product. There must be systems to provide documented proof that correct procedures are consistently followed at each step in the manufacturing process - every time a product is made.



COMPONENTS OF GMP

GMP requires that the manufacturing process is fully defined before being initiated and all the necessary facilities are provided. In practice, personnel must be adequately trained, suitable premises and equipment used, correct materials used, approved procedures adopted, suitable storage and transport facilities available, and appropriate records made. The essential components of GMP are summarized in Figure 1.





Fig.1. Components of Good Manufacturing Practice





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These regulations, which have the force of law, require that manufacturers, processors, and packagers of drugs, medical devices, some food, and blood take proactive steps to ensure that their products are safe, pure, and effective. GMP regulations require a quality approach to manufacturing, enabling companies to minimize or eliminate instances of contamination, mixups, and errors. This in turn, protects the consumer from purchasing a product which is not effective or even dangerous. Failure of firms to comply with GMP regulations can result in very serious consequences including recall, seizure, fines, and jail time.

SGMP regulations address issues including recordkeeping, personnel qualifications, sanitation, cleanliness, equipment verification, process validation, and complaint handling.



Most GMP requirements are very general and openended, allowing each manufacturer to decide individually how to best implement the necessary controls.

This provides much flexibility, but also requires that the manufacturer interpret the requirements in a manner which makes sense for each individual business.

SGMP is also sometimes referred to as "cGMP". The "c" stands for "current," reminding manufacturers that they must employ technologies and systems which are up-todate in order to comply with the regulation.

Systems and equipment used to prevent contamination, mixups, and errors, which may have been "top-of-the-line" 20 years ago, may be less than adequate by today's standards.



In the Drugs and Cosmetics Rules, 1945, for Schedule M, the following Schedule shall be substituted, namely: -

<u>PART – I</u>: Good Manufacturing Practices for Premises and Materials

- 1. General requirements
 - A. Location and surroundings
 - B. Buildings and premises
 - C. Water system
 - D. Disposal of waste
- 2. Warehousing Area
- 3. Production area
- 4. Ancillary areas
- 5. Quality Control area
- 6. Personnel
- 7. Health, clothing and sanitation of workers
- 8. Manufacturing Operations and Controls
- 9. Sanitation in the manufacturing premises
- 10. Raw materials



- 11. Equipment
- Documentation and records
- Labels and other Printed Materials
- Quality Assurance
- 15. Self inspection and Quality audit
- Quality Control System
- Specification
- Master Formula Records
- Packaging Records
- 20. Batch Packaging Records
- 21. Batch Processing Records
- 22. Standard Operating Procedures (SOPs) and Records, regarding
- 23. Reference samples
- 24. Reprocessing And Recoveries
- 25. Distribution records
- 26. Validation And Process Validation
- 27. Product recalls
- 28. Complaints and Adverse Reactions
- 29. Site Master File







Benefits of GMP Certification:

- Prove enterprise's management capabilities in product quality, safety assurance
- Enable employees to develop good production / operations habits
- Reduce safety risk in product quality and safety
- •Timely detect production and management problems, reduce cost
- •Better understand and comply with the relevant laws and regulations.
- •Enhance the international credibility and public image
- Increase customer's long-term confidence in the enterprise



THERAPEUTIC L-ASPARAGINASE PRODUCTION AND FORMULATION METHODOLOGY:

1. INTRODUCTION :

- L-asparaginase (EC3.5.1.1) catalyzes the hydrolysis of L-asparagine into aspartic acid and ammonia. L-asparaginase has been a clinically satisfactory antitumor agent for the valuable treatment of acute lymphoblastic leukemia (ALL) and lymph sarcoma.
- The microorganisms are a better source of L-asparaginase because they can be cultured easily . Erwinia caratovira, Corynebacterium glutamicum, Bacillus sp. Psudomonas stutzeri, and E. coli are most commonly used microorganisms for the production of L-asparaginase.

2. CLINICAL SIGNIFICANCE

L-asparaginase has been a clinically satisfactory antitumor agent for the valuable treatment of acute lymphoblastic leukemia (ALL) and lymph sarcoma. L-asparagine is an essential amino acid for the production of protein in tumor cells whereas the growth of normal cell is independent of its requirement.



L-asparaginase can be produced within the cells by an enzyme called asparagine synthase are can be absorbed from the outside. Lymphatic tumor cell required huge amount of asparagine to keep up their rapid malignant growth. In the presence of L-asparaginase tumor cell get deprivated and cannot survive. Asparagene is required for cell survival and DNA synthesis; however, most of the cells are capable to synthesizing asparaginase from glutamine. Acute lymphoblastic leukemia cells lack adequate level of the asparagines synthase and cannot survive



in asparagine depletion. Asparginase is cycle specific for the G1 of cell cycle

3. CULTIVATION METHOD

Solid State Fermentation :

(SSF) is suitable for the production of enzyme by using natural substrate because they mimic the condition under which the microbe grows natured. The solid state fermentation has several advantages over submerged fermentation including superior productivity, low capital investment, simple technique, low energy requirement less water output and better product recovery.

Rice bran served as a most appropriate substrate compared to other existing starchy materials, for solid stats cultivation of Serratia marcescens SBOB for L-asparaginase production.

Submerged Fermentation :

Production of L- asparaginase highly influenced by fermentation media composition and culture condition such as pH, temperature, agitation rate inoculums size, incubation time.



4. PURIFICATION STEPS :

Removal Of Insoluble Material :



the fermentation processes involved are always followed by the removal of insoluble materials, e.g., cells from the culture or cell debris from crude broth, either by centrifugation or by filtration. Centrifugation is a standard unit operation in some downstream recovery processes and is primarily used to separate solids from liquids. It can remove particles from as small as 0.5 µm to whole cells or organisms. Centrifugation is the process of choice for the removal of cells and/or cells debris. Filtration is used to remove small particles from solutions or to concentrate or separate soluble compounds.

Product Enrichment And Concentration :

Precipitation- The use of ammonium sulfate as a precipitation agent for L-ASN is well known; however, there is no standard protocol for L-ASNase precipitation. The use of organic solvents or short chain alcohol such as methanol and ethanol, for precipitation is well known. Although the selectivity obtained with precipitation is poor when compared with other purification techniques, such as chromatography. The main disadvantage, when compared with other low resolution techniques, is the likelihood of irreversible inactivation of the bioproduct during the precipitation process.

Recovery Of Biological Products:

Aqueous Two Phase System : ATPSs can be formed when two chemically different polymers (e.g. poly-ethylene glycol -PEG) and dextran) or one polymer and a specific salt (e.g., PEG and potassium phosphate) are mixed together at certain concentrations in a solution. One phase is rich in one polymer, and the second phase is rich in the other component (polymer or salt) with water as a solvent in both phases. Manipulating and optimizing the equilibrium compositions in each of the immiscible phases can induce differential partitioning behavior in a mixture. Knowledge of a higher affinity of certain compounds for one phase with respect to the other can be exploited for using phase partitioning for the purification of proteins. Although ATPS are easy to scale up, the very low solubility of proteins is a problem, possibly related to crowding effects in such aqueous systems.

High Resolution Purification :

After the first steps of purification, chromatographic steps are often used to achieve maximum purification and/or to polish the target compound. Selection of the procedure to use should be based on its capacity; the general rule is to proceed from a high to a low capacity method (i.e., from ion-exchange chromatography to affinity chromatography)

- i. Ion Exchange Chromatography : Based on the attraction of compounds with different surface charges, and a protein's surface charge dependent on its pl and the pH
- ij. Gel Filtration Chromatography : based on the fractionation of compounds according to their size
- iii. Affinity chromatography : based on the interaction of two compounds that bind to each other with high affinity, such as enzyme-substrate, receptor-ligand, and antigen-antibody interactions.
- iv. Aqueous Two-phase Micellar Systems : Surface-active agents are compounds that are typically composed of two chemically distinct portions: a hydrophilic portion and a hydrophobic portion. Due to their distinct chemical structures, when compounds of surfactants are dissolved in water, aggregate structures known as micelles form spontaneously.



In a micelle, the hydrophobic tails attract one another to minimize their unfavorable contact with water, while the hydrophilic heads remain on the outer surface of the micelle to maximize their



contact with water. At certain temperatures and concentrations of nonionic surfactants, a homogeneous micellar aqueous solution can be separated into two macroscopic phases, both containing micelles, but with one having a higher concentration of surfactant in reverse micelles. This phase separation is induced by a "temperature increase" of the system separation is induced by a "temperature increase" of the system up to reaching what is called the cloud point temperature.



WHAT ARE SINGLE CELL PROTEINS ?

- SCP are dried cells of micro organisms which can be used as dietary protein supplement.
- They are used as animal feed & can be used for human feed as protein supplement.
- Also called 'Novel Food' & 'Minifood'.







- Environmental Biotechnology may be referred as application of biotechnological techniques to solve the problems and issues of environment.
- Biomass as the name suggests consists of all organic matter that grows by photosynthetic conversion of solar energy.
- This biomass is available abundantly and is estimated to contain 3x 10²¹ joules of energy, some 10 times the yearly worldwide consumption.
- Utilization of Biomass would lead to
 - a. Help solve modern waste disposal problems.
 - b. Decrease in environmental pollution.
 - c. Help activate shortage of food and animal feed.



HISTORY

- Part of our diet since ancient times.
- Earlier known as 'Microbial Protein'.
- Name was introduced by Prof. Scrimshow of MIT in 1967
- In 1950's British Petroleum initiated production of SCP on commercial basis.
- Pruteen was the 1st commercial SCP used as animal feed additive
- Pruteen was produced from bacteria <u>Methylophilus</u> <u>methylotrophus</u> cultured on methanol & had 72 % protein content.



SCP PRODUCTION IN INDIA

- National Botanical Research Institute (NBRI).
- Central Food Technological Research Institute (CFTRI).
- In CFTRI, SCP is produced from algae cultured on sewage.



RAW MATERIALS

- Production of SCP requires micro-organisms that serve as the protein source and the substrate that is biomass on which they grow.
- There is a variety of both the sources that can be used for the production of SCP.
- The biomass used can be plant biomass or organic biomass.
- The micro-organisms used belong to the group of Algae, Fungi and Bacteria.



MICRO ORGANISMS

•Micro-organisms used are fungi , yeast, algae & bacteria. •The following table shows average different compositions of main groups of micro- organisms (% dry wt.)

COMPOSITON	FUNGI	ALGAE	YEAST	BACTERIA
PROTEIN	30- 40 %	40- 60 %	45- 55 %	50- 65 %
FAT	9-14 %	8-10 %	5-10 %	3-7 %
NUCLEIC ACID	7-10 %	3-8 %	6-12 %	8-12 %



A list of the micro-organisms used for SCP production

Fungi

- Aspergillus fumigatus
- Aspergillus niger
- Rhizopus cyclopium

Yeast

- Saccharomyces cerevisae
- Candida tropicalis
- Candida utilis

Algae

- Spirulina sps.
- Chlorella pyrenoidosa
- Chondrus crispus

Bacteria

- Pseudomanas fluraescens
- Lactobacillus
- Bacillus megaterium



FUNGI

YEAST



ALGAE



BACTERIA



COMPARISION OF MICRO-ORGANISMS

	ADVANTAGES	DIS ADVANTAGES	
FUNGI	Easy to grow & harvest	Lower growth rates & lower protein content	
ALGAE	Easy to grow & harvest & high quality protein	Non –digestible cellulosic cel wall, concentrate heavy metals	
YEAST	Larger in size, lower NA content , familiarity & acceptability	Poor digestibility, low protein content, slow growth rate	
BACTERIA	High protein content, digestible cell wall	High NA content, small in size, low density	



Biomass

- Biomass also plays a very important role in the production of SCP.
- Selection of biomass depends on the micro-organisms used for the production.
- For eg. Algae are cultivated on sewage whereas Yeast are cultured on agro-industrial wastes.

Algal Biomass

- Algae grows autotropically.
- Requires low intensity of light.
- Temperature 35 40
 C & pH 8. 5 10.5
- Cultivated in large trenches of sewage oxidation ponds.





Bacterial & Fungal biomass

- Bacteria & fungi can be grown easily on a wide range of substrates.
- They require a minimum temperature of 15º-34ºc & a pH of 5-7.





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Yeast biomass

- Cultivated on agro- industrial wastes such as molasses, starchy materials, fruit pulp, wood pulp, etc.
- Requires a temperature of 30 -34
 c & pH of 3.5-4.5.
- Also requires addition of inorganic acids & sulphur supplements in the form of salts.





FACTORS AFFECTING BIOMASS PRODUCTION

- Illumination time
- Temperature
- .pH
- Suitable strains
- Agitation
- Sterile conditions



SCP PRODUCTION

- Selection of suitable strain
- Fermentation
- Harvesting
- Post harvest treatment
- SCP processing for food

Selection of strain

- It a very critical step as the quality of protein depends totally on the • microbe that is used for the production.
- Thus careful selection of the strain should be done.
- Care should be taken that the selected strain should not produce any • taxic or undesirable effects in the consumer.

Fermentation

- It can be carried out in the fermentor which is equipped with aerator, ٠ thermostat, pH, etc. or in the trenches or ponds.
- Microbes are cultured in fed- batch culture.
- Engineers have developed deep lift fermentor & air lift fermentor .









Harvesting

- When the colonies of microbes are fully developed, they are then harvested.
- The bulk of cells are removed from the fermentor by decantation.

Post harvest treatment

- After harvesting, the cells are subjected to a variety of processes.
- Post harvesting treatments includes steps like separation by centrifugation, washing, drying, etc.





PROCESSING FOR FOOD

lt includes

- Liberation of cell proteins by destruction of indigestible cell wall.
- A. MECHANICAL METHODS
- Crushing, crumbling, grinding, pressure homogenization, etc.
 B. CHEMICAL METHODS
- Enzymes & salts are used to digest or disrupt the cell wall.
- Salts like NaCl, sodium dodecyl sulfate, etc. whereas nuclease enzymes are used.
- C. PHYSICAL METHODS
- Freeze- thaw, osmotic shock, heating & drying.



2. Reduction of nucleic acid content

- Chemical & enzymatic treatments are preferred.
- Chemicals which are used includes acidified alcohol, salts, acids 8 alkalies.
- Use of such chemicals leads to formation of lygino-alanine which causes hypersensitivity skin reactions.
- Enzymes which are used include ribonuclease & nuclease enzymes .
- These enzymes can be used exogenously or can be induced endogenously.




ADVANTAGES

- Rapid successions of generations.
- Easily modifiable genetically.
- High protein content of 43-85% in dry mass.
- Broad spectrum of original raw material used for production, which also includes waste products.
- Production in continuous cultures
- consistent quality not dependent on climate in determinable amount
- low land requirements, economically beneficial.
- Utilization of solar energy
- Cellular, molecular and genetic alterations.



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DISADVANTAGES

- High content of nucleic acids leading to elevated levels of unic acid.
- Development of kidney stone and gout if consumed in high quality.
- Possibility for the presence of secondary toxic metabolites.
- Poor digestibility
- Stimulation of gastro-intestinal
- Hypersensitivity skin reactions.



APPLICATIONS

- 1. As protein supplemented food-
- Also source of vitamins, amino acids, minerals, crude fibers, etc.
- Supplemented food for undernourished children.

2. As health food-

- Controls obesity
- Provides instant energy .
- Example- Spirulina- part of diet of US Olympic team.





- 3. In therapeutic and natural medicines-
- Reduce body weight, cholesterol, stress.
- Lowers blood sugar level in diabetic(due to presence of B linolenic acid)
- Prevents accumulation of cholesterol in body.
- Healthy eyes and skin (beta carotene)
- Beta carotene (anti cancer substance-**UN National Cancer Research Institute)**
- Increase lactation.





4. In cosmetics-

- Important role in maintaining healthy hair (vitamin A and B).
- Many herbal beauty products.
- Biolipstics and herbal face cream(Phycocyanin).
- Capable of replacing coal tar dye based cosmetics.

5. Poultry and cattle feed-

- Excellent, convenient source of protein and other nutrients.
- Used to feed cattle, fishes etc.







CONCLUSION

- At present SCP production is in its infancy. One of the ways to enhance productivity and quality is genetic improvements of micro-organisms.
- Using microbial biomass as a food source deserves serious consideration because of insufficient world food supply and high protein content of most microorganisms.

