Chapter 6

Industrial Microbiology

Learning Objectives

After studying this chapter the students will be able to,

- *Know the concepts involved in industrial microbiology and the production of industrially important products by microorganisms.*
- *Understand the primary and secondary screening process.*
- *Gain knowledge in the field of strain improvement of microorganisms.*
- *Describe the structure components and function of a fermentor.*
- *Known the principles behind fermentation medium, fermentation process, upstream processing and downstream processing.*
- *Know the values of microorganism used in the production of penicillin citric acid, wine and single cell protein.*
- *Analyze the basics behind immobilization of microorganisms*

Chapter Outline

6.1 Industrially Important Microorganisms and their Products

6.2 Screening of Industrially Important Microorganism

6.3 Strain Improvement

6.4 Preservation of Industrially Important Microorganisms

6.5 Fermentors

6.6 Industrial Production of Penicillin

6.7 Industrial Production of Wine

6.8 Industrial Production of Single Cell Protein

6.9 Industrial Production of Citric Acid

6.10 Immobilization

Industrial microbiology is a branch of science that deals with the study and uses of various microorganisms that are responsible for the production of many products which has industrial and economic applications. Man has been using many microorganisms for the production of foods, (bread, cheese, yogurt, pickles)–beverages (beer, wine) for many centuries. The birth of industrial microbiology largely began with the studies of Pasteur on fermentation. The term Fermentation originates from a Latin verb "Fervere" which literally means to boil. In alcohol production, CO_2 (gas bubbles) Figure 6.1 are formed during boiling of liquid.

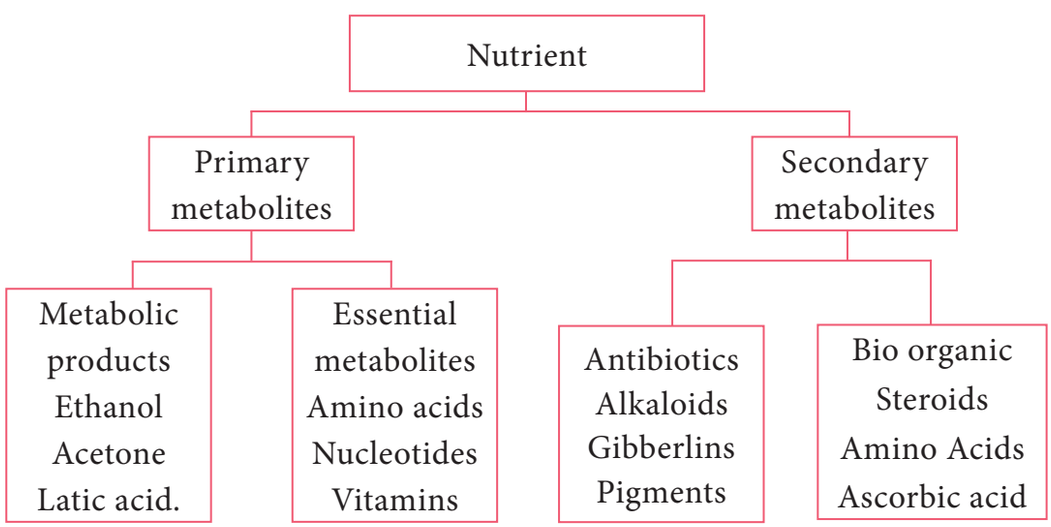
Figure 6.1: Bubble formation in grape juice fermentation

6.1 Industrially Important Microbes and their Products

Microorganisms have the powerful capacity to produce numerous products, during their life cycle. Flowchart 6.1 shows the production of valuable metabolic products during the growth of microorganisms on a suitable medium under controlled environmental conditions. Microbial products are often classified as primary and secondary metabolites.

Primary metabolites consist of compounds related to the synthesis by microbial cells in the growth phase. Primary metabolites such as amino acids, vitamins, enzymes, organic acids and nitrogenous bases are produced by wide variety of microorganisms. These primary metabolites are essential for the growth of microorganisms and they are produced during Logarithmic phase. Secondary metabolites do not play a role in development, growth and reproduction of microorganisms. They are produced at the end of growth phase near stationary phase. They usually accumulate during the period of nutrient limitation or waste product accumulation that follows the exponential phase. These compounds have no direct relationship to the synthesis of cell materials and normal growth. They are the end products of the primary metabolism. Products such as steroids, alkaloids, antibiotics are secondary metabolites.

Excessive production of the primary and secondary metabolites produced by the microorganisms are useful in the large scale in industrial production. Unlike primary metabolites, secondary metabolites are produced in small quantities and their extraction is difficult (Figure 6.2).



Flowchart 6.1: Various metabolites produced in Industrial fermentation

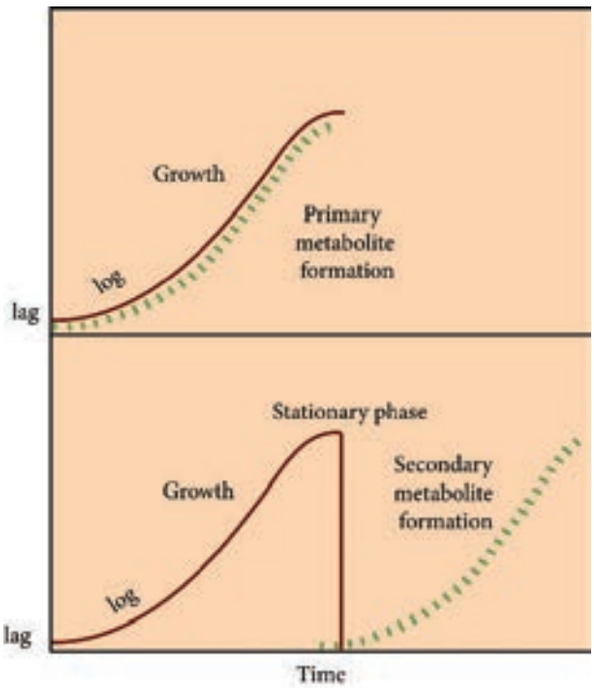


Figure 6.2: Production of primary and secondary metabolites in the growth cycle of microorganism

Some industrially important products are,

- microbial cells (living or dead), microbial biomass and components of microbial cells
- microbial metabolites
- intracellular or extracellular enzymes
- modified compounds that has been microbiologically transformed, and
- recombinant products through the DNA recombinant technology. (Table 6.1 shows some industrially important microorganisms)

The nutritional yeast is called food yeast. The yeast cells are killed during manufacturing, and not alive in the final product. It is used in cooking; it has a cheesy, nutty or savory flavour. Yeast S. *cerevisiae* is used as food yeast. It is a vegan food, available in both fortified (with some vitamins) and unfortified form.

The industrial production of commercial products is carried out by fermentation process. The term fermentation is defined scientifically in a strict sense as a biological process that occurs in the absence of oxygen (anaerobic). In industrial sense any process mediated by or involving microorganisms in which a product of economic value is obtained is called fermentation. The term Industrial fermentation also means large scale cultivation of microorganisms even though most of them are aerobic.

Table 6.1: Industrially important microorganisms

Product	Microorganisms	Uses
Vitamin B12	*Streptomyces*	Vitamin supplements
Lactic acid	*Lactobacillus delbrueckii*	Chemical reagents
Citric acid	*Aspergillus niger*	Food preservative
Acetic acid	*Acetobacter*	Vinegar, solvent
Ethanol	*Saccharomyces*	Chemical reagents drinks
Penicillin	*Pencillium chrysogenum*	Antibiotic

There are many microbiological processes that occur in the presence of air (aerobically) yielding incomplete oxidation products. Examples: i) the formation of acetic acid (vinegar) from alcohol by vinegar bacteria ii) citric acid from sugar by certain molds such as Aspergillus niger. These microbial processes are often referred to as fermentations, although they do not decompose in the absence of air.

Infobits

The German Eduard Buchner, winner of the 1907 Nobel Prize in chemistry, determined that fermentation was actually caused by a yeast secretion that he termed zymase. The experiment for which Buchner won the Nobel Prize consisted of producing a cell–free extract of yeast cells and showing that this "press juice" could ferment sugar. This finding dealt yet another blow to vitalism by demonstrating for the first time that fermentation could occur outside living cells.

6.2 Screening of Industrially Important Microorganism

Isolation of industrially important microorganisms

Success of fermentation depends upon the isolation of microorganism. The microorganisms are isolated from their natural habitats like soil, lakes, river mud or even in unusual habitats or environments such as extreme cold, high altitude, deserts, and deep sea and petroleum fields and are tested directly for the product formation and isolated or it can be genetically modified. Different types of microorganisms are isolated by different methods.

Different microbes with desired activity are isolated using various culture techniques. The next step after isolation of microorganisms is the selection or screening. For the successful fermentation process, selection of microorganisms is the prime important step. Screening includes primary screening and secondary screening.

Primary screening: The elementary steps that are performed to select the desired organisms and eliminate the undesirable organisms are termed as primary screening. Methods such as crowded plate technique, auxanography and enrichment culture technique are some of the techniques used in primary screening. For screening of antibiotic producing organisms crowded plate technique is described here,

Crowded plate technique

1. Soil is serially diluted
2. The serially diluted sample is spread on the nutrient agar plates
3. The plates are incubated and the agar plate having 300 to 400 colonies are observed for antibiotic producing activity
4. The ability of a colony to exhibit antibiotic activity is indicated by the presence of a zone of inhibition surrounding the colony
5. The technique is improved by using test organism
6. The antibiotic produced by the organisms in the soil may inhibit the growth of test organism

7. The formation of inhibitory zones around certain colonies indicates their antibiotic sensitivity
8. The diameter of the zones of inhibition is measured in millimeters. Crowded plate technique is depicted in the diagram (Figure 6.3).

Enrichment isolation

The process of enrichment provides a suitable condition to support the growth of microorganisms. It allows the growth of the specific microbe while inhibiting the other non-target microbe. The growth of target microorganisms is enriched by providing sole carbon source. For screening microorganisms degrading the compound, different inhibitors are employed which have the ability to block a specific metabolic pathway of the non–target microbe. pH and temperature are also adjusted favoring the growth of desired microorganisms. Soil Calcium carbonate enrichment technique is used for isolation of secondary metabolite producing microorganisms (actinomycetes).

Secondary screening

It is very useful in sorting out microorganisms that have real commercial value from many isolates obtained during primary screening.

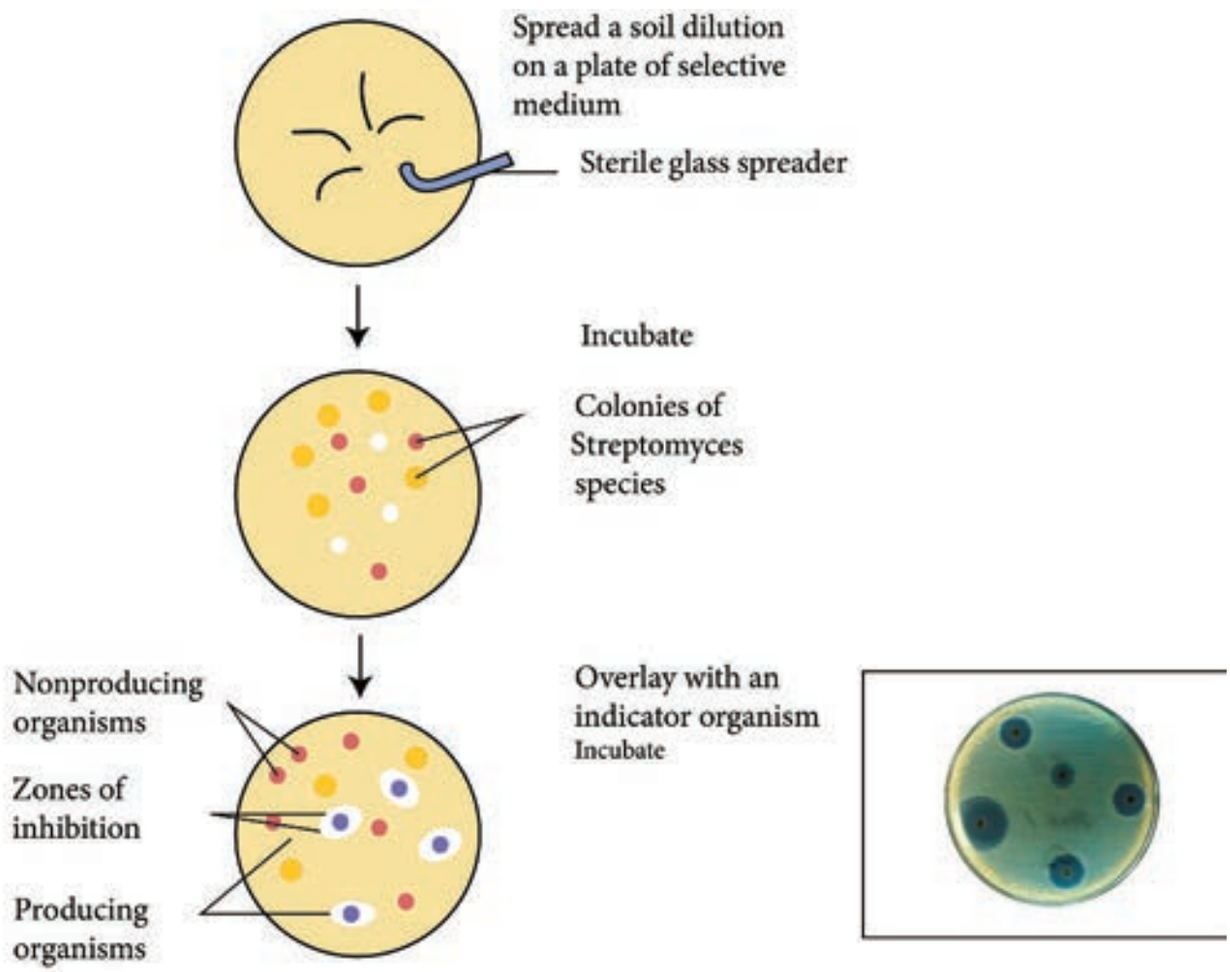


Figure 6.3: Crowded plate technique

HOTS

Why microorganisms are exploited more than plant and animal cells for production of commercial products?

1. As primary screening allows the detection and isolation of microorganisms which posses, potentially interesting industrial applications. It is further followed by secondary screening, to check the capabilities and gain information about these organisms.
2. Through primary screening only few or many microorganism that produce a industrially important product, are isolated. The information about the product formed is very less. So, through secondary screening, further sorting out is performed. In this method, only microorganisms with real commercial value are selected and those that lack the potential are discarded.
3. Secondary screening should yield the types of information which are needed in order to evaluate the true potential of a microorganisms industrially usage.
4. Secondary screening may be qualitative and quantitative in its approach.
5. It is done by using paper, thin layer or other chromatographic techniques.
6. The product's physical, clinical, and biological properties are determined.
7. It detects gross genetic instability in microbial cultures.
8. It gives information about the number of products produced in a single fermentation.
9. It determines the optimum conditions for growth or accumulation of a product associated with particular culture.
10. It gives information about the different components of the fermentation medium.
11. It helps in providing information regarding the product yield potential of different isolates.
12. It reveals whether microorganisms are capable of a chemical change or it destroys their fermentation product.

There are various methods employed for secondary screening which includes test conducting on petridish containing solid media or by using flasks or small fermentors containing liquid media, giant colony technique, and filtration method liquid medium method (using Erlenmeyer flask). Here giant colony technique is explained in detail.

Giant Colony Technique

The Streptomyces culture is inoculated onto the central areas of petriplates containing a nutritious agar medium or they are streaked in a narrow band across the centre of plates. The plates are then incubated until growth and possibly, sporulation have occurred. Strains of micro organisms to be tested for possible sensitivity to the antibiotics (the test organisms) are then streaked from the edges of the plates up to but not touching the *Streptomycete* growth. The plates are further incubated to allow the growth of the test organism. The growth of the

test organism inhibited by antibiotic in the vicinity of the *Streptomycete* is then measured in millimeters. These *Streptomycetes* that have produced antibiotics with observable microbial inhibition spectrum are retained for further testing (Figure 6.4).

The microbes used in the industrial microbiology should have following characters.

1. The strain should be a high–yielding strain.
2. The strain should have stable biochemical and genetical characteristics.

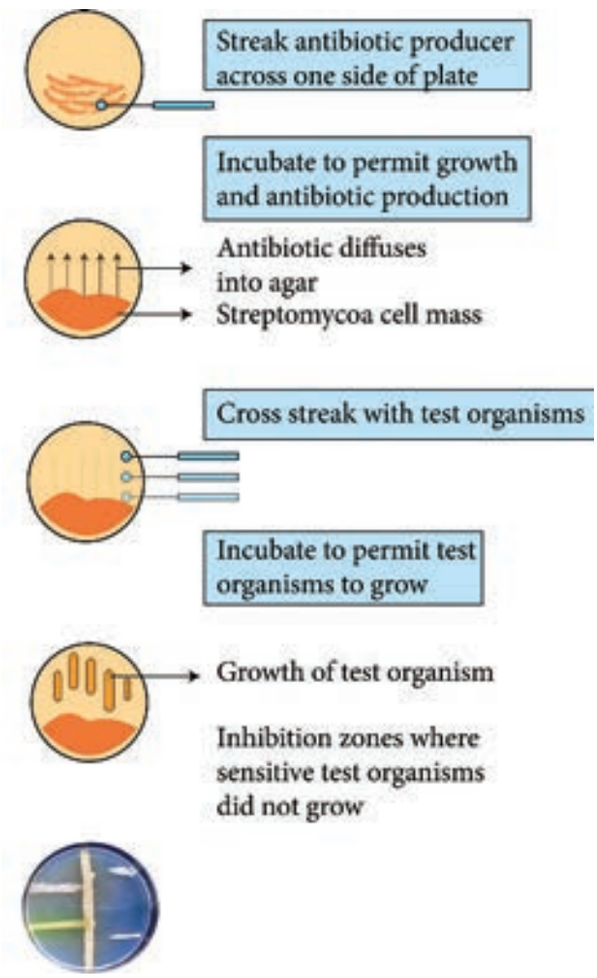


Figure 6.4: Giant Colony Technique

3. It should not produce undesirable substances.
4. It should be easily cultivated on large scale.

The strain should be in pure culture, free from other microorganisms including Bacteriophages. These characters are screened for the production of desirable products from microorganisms.

6.3 Strain Improvement

Improvement of the production strain(s) offers the great opportunities for cost reduction without significant capital outlay in industries. Moreover, success in making and keeping a fermentation industry competitive depends greatly on continuous improvement of the production strain(s). Improvement usually resides in increased yields of the desired metabolite. The science and technology of manipulating and improving microbial strains, in order to enhance their metabolic capacities for biotechnological applications, are referred to as strain improvement.

Need for strain improvement

Microbes exist in the nature produce certain compounds of biological interest. However the industrial application of producing those compounds by natural strains is not an economical one so, wild strains are changed by the changing their gene pattern or by regulating their enzymes production. As a result, the specific product is produced in excess.

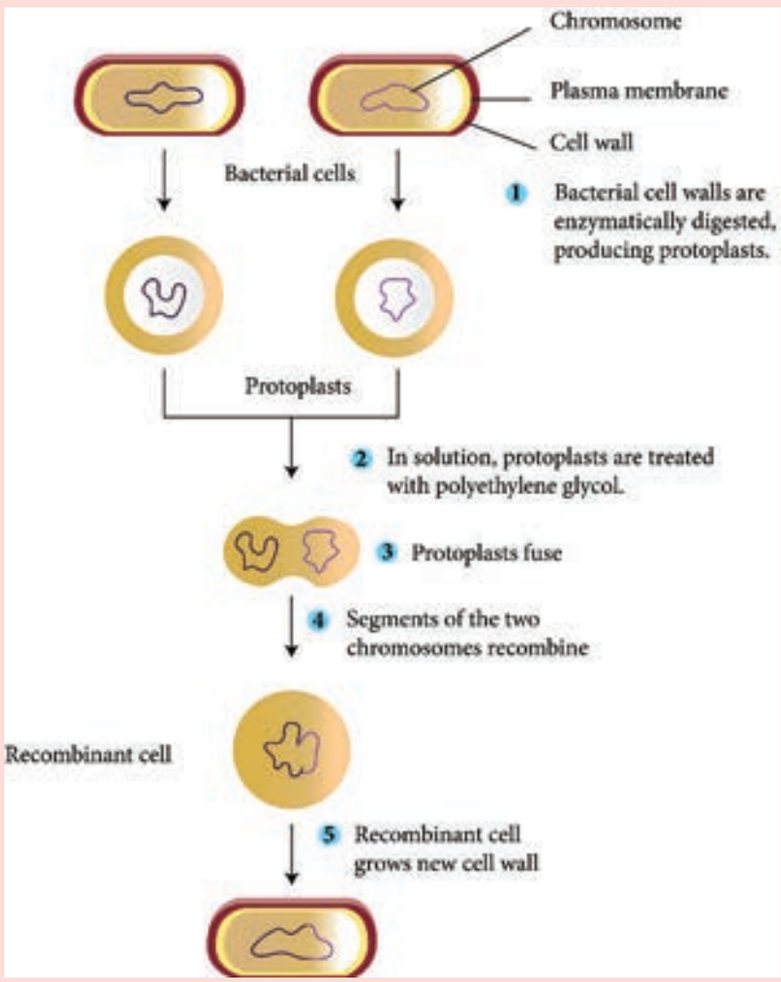
Knowledge of the function of enzymes, rate limiting steps in pathways, and environmental factors controlling synthesis further helps in designing screening strategies.

Attributes of Improved strains

1. Assimilate inexpensive and complex raw materials efficiently.
2. Alter product ratios and eliminate impurities or by products in downstream processing.
3. Reduce demand on utilities during fermentation (air, cooling water, or power).
4. Provide cellular morphology in a form suitable for product separation.
5. Create tolerance to high product concentration.
6. Shorten fermentation times.
7. Overproduce natural products or bioactive molecules not synthesized naturally for example insulin.
8. Excrete the product to facilitate product recovery.

Generally wild strains of microorganisms produce low quantities of commercially important metabolites. So, genetic improvements have to be made and new strains need to be developed for any substantial increase in the product formation in a cost effective manner.

Protoplast fusion is defined as fusion of two different protoplasts. A cell wall less nature of plant, bacteria, fungal is called protoplast. It is re moved by either mechanical or enzymatic means. Protoplast has nucleus other protoplasmic contents which are surrounded by cytoplasmic membrane.



HOTS

An organism is isolated from soil, which is a very low yielding one. How will you enhance the production activity?

The following techniques at practical genomic level help to improve the microbial strain. They are:

1. Selection of mutants
2. Recombination
3. Regulation
4. Genetic engineering
5. Protoplast fusion

6.4 Preservation of Industrially Important Micro Organisms

The selected microorganism of industrial interest must be preserved in its original form for any further use and research. There are different methods for microbial preservation. Suitable methods are selected based on the:

a. Type of micro-organism

b. Effect of the preservation method on the viability of micro-organism

c. Frequency at which the cultures are withdrawn

d. Size of the microbial population to be preserved

e. Availability of resources

f. Cost of the preservation method. Followings are some of the methods of microbial preservation:

a. Desiccation

This involves removal of water from the culture. Desiccation is used to preserve actinomycetes (a form of fungi-like bacteria) for very long period of time. The microorganisms can be preserved by desiccating on sand, silica gel, or paper strips.

b. Agar Slopes

Microorganisms are grown on agar slopes in test tubes and stored at 5 to –20 °C for six months. If the surface area for growth is covered with mineral oil the micro-organisms can be stored for one year.

c. Liquid Nitrogen

This is the most commonly used technique to store micro-organisms for a long period. Storage takes place at temperatures of less than -196 °C and even less in vapour phase. Microorganisms are made stationary and suspended in a cryoprotective agent before storing in liquid nitrogen.

d. Drying

This method is especially used for sporulating microorganisms (organisms that produce spores). They are sterilized, inoculated, and incubated to allow microbial growth, then dried at room temperature. The resultant dry soil is stored at 4° to 5 °C.

e. Lyophilization

This process is also known as freeze-drying. The microbial culture is first filled in ampoules (glass vessels) and frozen, then dried under vacuum. This is a most convenient technique, since it is cheap to store and easy to ship. The disadvantage is that it is difficult to open the freeze dried ampoules; also, several subcultures have to be done to restore the original characteristics of the microorganisms.

6.5 Fermentors

The main function of a fermenter is to provide a suitable environment in which an organism can efficiently produce a target product. Most of them are designed to maintain high biomass concentrations, which are essential for many fermentation processes. Fermentor design, quality of construction, mode of operation and the level of sophistication largely depend upon the production organism, the optimal operating conditions required for target product formation, product value and the scale of production. The performance of any fermenter depends on many factors, but the key physical and chemical parameters that must be controlled are agitation rate, oxygen transfer, pH, and temperature and foam production.

HOTS

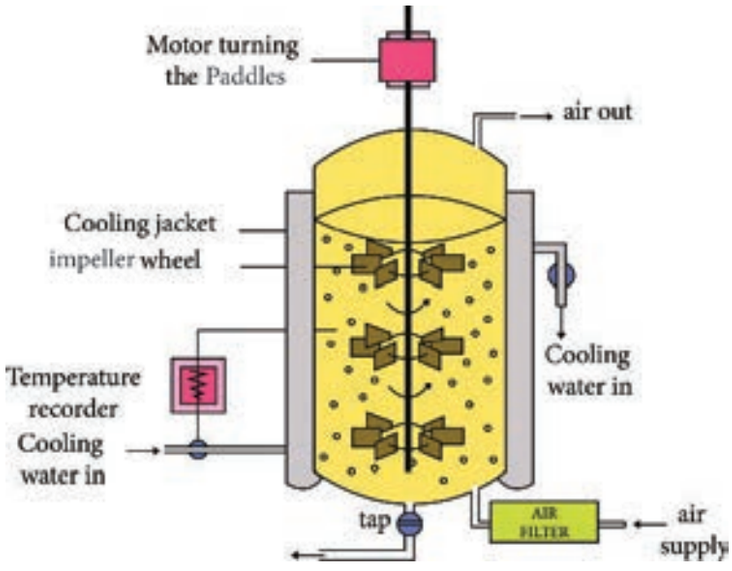
What will happen if antifoam agents are not used in the Fermentation process?

6.5.1 Basic Design of a Fermenter

The materials used for construction of fermenter withstand repeated steam sterilization and are nontoxic. The reaction vessel is designed to withstand vacuum or else it may collapse while cooling. The internal surface is smooth and corrosion resistant. Either stainless steel or glass is used for construction.

Conventional bioreactors are cylindrical vessels with dome top and bottom (Figure 6.5).

It is surrounded by a jacket and sparger at the bottom through which air is introduced. The agitator (for mixing of cells and medium) shaft is connected to a motor at the bottom. It has ports for pH, temperature, dissolved Oxygen sensors for regulation. Antifoam agents like animal vegetable oil, lard oil, corn oil and soya bean oil are used to control the foam. Modern fermentors are usually integrated with computers for efficient process monitoring and data acquisition. Parts of the fermenter and their functions are given in Table 6.2.



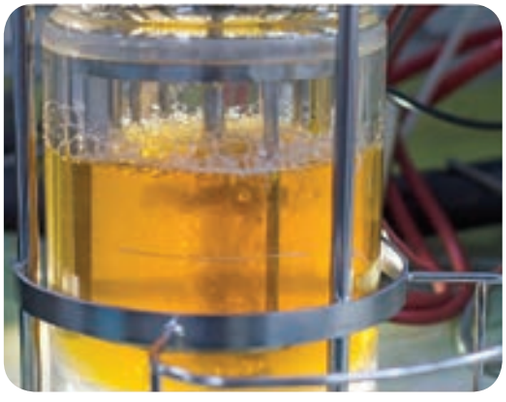


Figure 6.5: Design of a fermenter

Table 6.2: Components of fermenter and their uses:

S. no	Parts of fermenter	Functions
1	Impeller (agitator)	To stir the media continuously and hence prevent cells from settling down and distribute oxygen throughout the medium. Impeller speed decreases as the size of the fermenter increases
2	Sparger (aerator)	Introduce sterile oxygen to the media in case of aerobic fermentation process
3	Baffles (vortex breaker)	Disrupt vortex and provide better mixing
4	Inlet Air filter	Filter air before it enter the fermenter
5	Exhaust Air filter	Trap and prevent contaminants from escaping
6	Rota meter	Measure flow rate of Air or liquid
7	Pressure gauge	Measure pressure inside the fermenter
8	Temperature probe	Measure and monitor change in temperature of the medium during the process
9	Cooling jacket	To maintain the temperature of the medium throughout the process
10	pH probe	Measure and monitor pH of the medium
11	Dissolved oxygen probe	Measure dissolve oxygen in the fermenter
12	Level probe	Measure the level of medium
13	Foam probe	Detect the presence of the foam
14	Sampling point	To obtain samples during the process
15	Valves	Regulates and controls the flow of liquids and gases

There are different types of fermentor used in industrially micro biology which includes.

1. Stirred tank bioreactor
2. Tower bioreactors
3. Air lift bioreactors
4. Packed–bed bioreactors
5. Fluidized bed bioreactors
6. Photo bioreactors

6.5.2 Media Used in the Industrial Productions

Fermentation Medium

Most fermentation requires liquid media, often referred to as broth, although some solid-substrate fermentations are also operated. Fermentation media must satisfy all the nutritional requirements of the microorganism and fulfill the technical objectives of the process. Animal fats and plant oils are also incorporated into some media, often as supplements to the main carbon source.

Medium used for large scale production should have the following characteristics.

1. It should be cheap and easily available.
2. It should maximize the growth of the microorganism productivity and the rate of formation of the desired product.
3. It should minimize the formation of undesired products.

It should contain carbon source, nitrogen source, energy source, micro nutrients required for the industrial production. Table 6.3 shows common substances used in the industrial fermentation process.

Waste products from other industrial processes such as molasses, ligno cellulosic waste, and corn steep liquor are generally used as substrates for industrial fermentation.

Apart from carbon and nitrogen sources, some other components like minerals, vitamins, growth factors are also used in Industrial fermentations.

Minerals

Normally, sufficient quantities of cobalt, copper, iron, manganese, molybdenum, and zinc are present in the water supplies, and as impurities in other media ingredients. For example, corn steep liquor contains a wide range of minerals that will usually satisfy the minor and trace mineral needs.

Vitamins and growth factors

Many bacteria can synthesize all necessary vitamins from basic elements. For other

Table 6.3: Some common substrates used in the industrial fermentation process:

Carbon source	
Molasses	It is a byproduct of sugar industry. It is a cheap source of carbohydrates It also contains nitrogenous substances, vitamins, trace elements. (Example:) sugar cane, beetroot molasses
Malt extract	It is an aqueous extract of malted barley
starch, dextrin cellulose	They can be metabolized by microorganism. They used for the industrial production of alcohol
Whey	It is a byproduct of dairy industry used in the production of alcohol, SCP, vitamin B12, lactic acid, gibberllic acid
Methanol ethanol	Methanol in the cheapest substrate. It is utilized only by few bacteria yeast. Methanol is used for SCP. Ethanol is used for acetic acid production
Hydro molasses	It is a byproduct in glucose production from corn
Sulphate waste liquor	It is a spent sulfite liquor form the paper pulping industry. It is used in the production of ethanol by *Saccharomyces cerevisiae*, and in the growth of *Torula utilis* as a feed
Nitrogen sources	
Inorganic: Ammonium salts and ammonia	It is a cheap source of nitrogen
Urea (Organic)	It is a good and cheap source of organic source
Corn steep liquor (Organic)	It is formed during starch production from corn. It is rich in several amino acids
Yeast extract	It is rich in amino acids, peptides vitamins
Soy meal	It is a left out residue on preparing soybean oil from soybean seeds. It is used in antibiotic production
Peptones	The proteins hydrolysates are called as peptones. The source of peptones includes meat, cotton seeds and sunflower seeds

bacteria, filamentous fungi and yeasts, they must be added as supplements to the fermentation medium. Most natural carbon and nitrogen sources also contain at least some of the required vitamins as minor contaminants. Other necessary growth factors, amino acids, nucleotides, fatty acids and sterols, are added either in pure form or, for economic reasons, as less expensive plant and animal extracts.

Precursors

Some fermentation must be supplemented with specific precursors, notably for secondary metabolite production. When required, they are often added in controlled quantities and in a relatively pure form. Examples: Phenyl acetic acid or phenylacetamide added as side chain precursors in penicillin production.

6.5.3 Large Scale Production

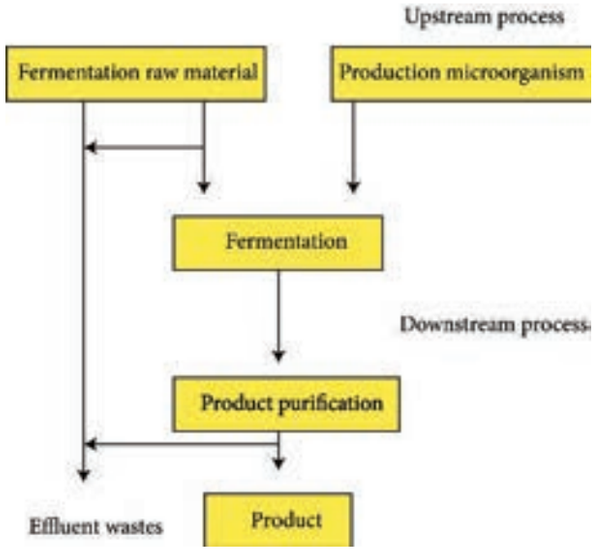


Figure 6.6: An overview of Fermentation process

Basic Steps of Industrial Fermentation

Successful development of a fermentation process and fermentors requires major contributions from a wide range of other disciplines, particularly biochemistry, genetics, molecular biology, chemistry, chemical engineering and process engineering, mathematics and computer technology. A typical operation involves both upstream processing (USP) and downstream processing (DSP) stages (Figure 6.6).

6.5.4 Upstream Processing

It is the first step in which biomolecules like bacteria or other cells are grown in a fermentor. Upstream processing involves inoculation development, scale up, medium preparation and sterilization of media and fermentation process.

Inoculum development

It is a preparation of a population of micro organisms from a stock dormant culture to a state useful for inoculating a final production fermentor.

It is a critical stage in fermentation process.

It is a stepwise sequence employing increasing volume of media.

Inoculum media is usually balanced for rapid cell growth and not for product formation.

Inoculum scale up

It is the preparations of the seed culture in amounts sufficient to be used in the larger fermenter vessel. It involves growing the microorganisms obtained from the pure stock culture in several consecutive fermenter. By doing this, the time required for the growth of microbes in the fermenter is cut down, so that the rate of productivity is increased. The seed culture obtained is then used for inoculation in

fermentation medium. The size of the inoculums is generally 1–10% of the total volume of the medium.

In general, fermentation/ bioprocess techniques are developed in stages starting from a laboratory and finally leading to an industry. The phenomenon of developing industrial fermentation process in stages is referred to as scale–up. Scale–up is necessary for implementing new fermentation technique developed using mutant organisms.

The very purpose of scale–up is to develop optimal environmental and operating conditions at different levels for a successful fermentation industry where conditions like substrate concentration agitation and mixing, aeration, power consumption and rate of Oxygen transfer are studied. In a conventional scale–up, a fermentation technique is developed in 3–4 stages. The initial stage involves a screening process using Petri dishes or Erlenmeyer flasks followed by a pilot project to determine the optimal operating conditions for a fermentation process with a capacity of 5–200 litres. The final stage involves the transfer of technology developed in the laboratory to industry. (Figure 6.7)

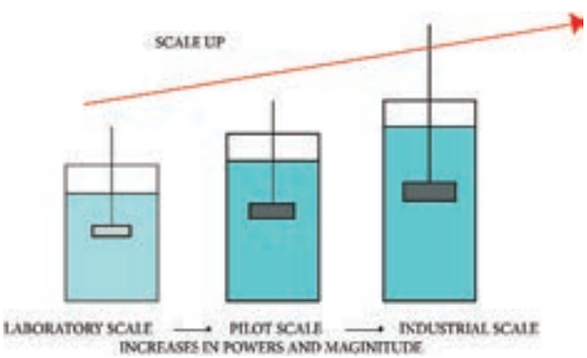


Figure 6.7: Scale up in industrial fermentation

It has to be continuously noted that a fermentation process that works well at the laboratory scale may work poorly or may not work at all on industrial scale. Therefore it is not always possible to blindly apply the laboratory conditions of a fermentation technique developed to industry.

At the laboratory scale, one is interested in the maximum yield of the product for unit time. At the industry level, besides the product yield, minimal operating cost is another important factor for consideration.

Preparation and sterilization of media

According to the specific industrial production basic components needed to carry out fermentation are selected as per the required volume.

Medium components should be free from contamination. So all the medium components employed in the fermentation process are sterilized. Sterilization is mostly carried out by applying heat and to lesser extent other physical methods, chemical methods (disinfectants) and radiation (using UV rays, γ rays). Batch Sterilization is carried out at 121°C (20 to 60 mins) where as continuous sterilization is done at 140°C for (30 to 120 secs). Much energy is wasted on batch sterilization on compared with continuous sterilization nearly 80 to 90% of energy saved during this process. Air and heat sensitive components are sterilizied by membrane filters.

Fermentation Process

It involves the propagation of the microorganism and the production of the desired product. Fermentation process is divided depending on the feeding strategy of the culture and medium as follows.

i. Batch Fermentation

ii. Continuous Fermentation

iii. Fed batch Fermentation

i. Batch Fermentation

The medium and culture are initially fed into the vessel and it is then closed. After that, no components are added apart from Oxygen. The pH is adjusted during the course of process by adding either acid or alkali. The fermentation is allowed to run for a predetermined period of time and the product is harvested at the end. Foaming is controlled by adding antifoam agents such as palm oil or soybeans oil. Heat generated is regulated by providing water circulation system around the vessel for heat exchange.

ii. Continuous fermentation

This is an open system. It involves the removal of culture medium continuously and replacement of them with a fresh sterile medium in a bioreactor.

In this method, homogenously mixing reactors which include chemo stat and turbid stat bioreactors are used. Examples: production of antibiotics, organic solvents, beer, ethanol and SCP.

iii. Fed batch system

It is a combination of both batch and continuous systems. In this, additional nutrients are added to the fermentors as the fermentation is in progress. This extends the time of operation, but the products are harvested at the end of the production cycle as in batch fermenter.

HOTS

Why does industry prefer continuous culture?

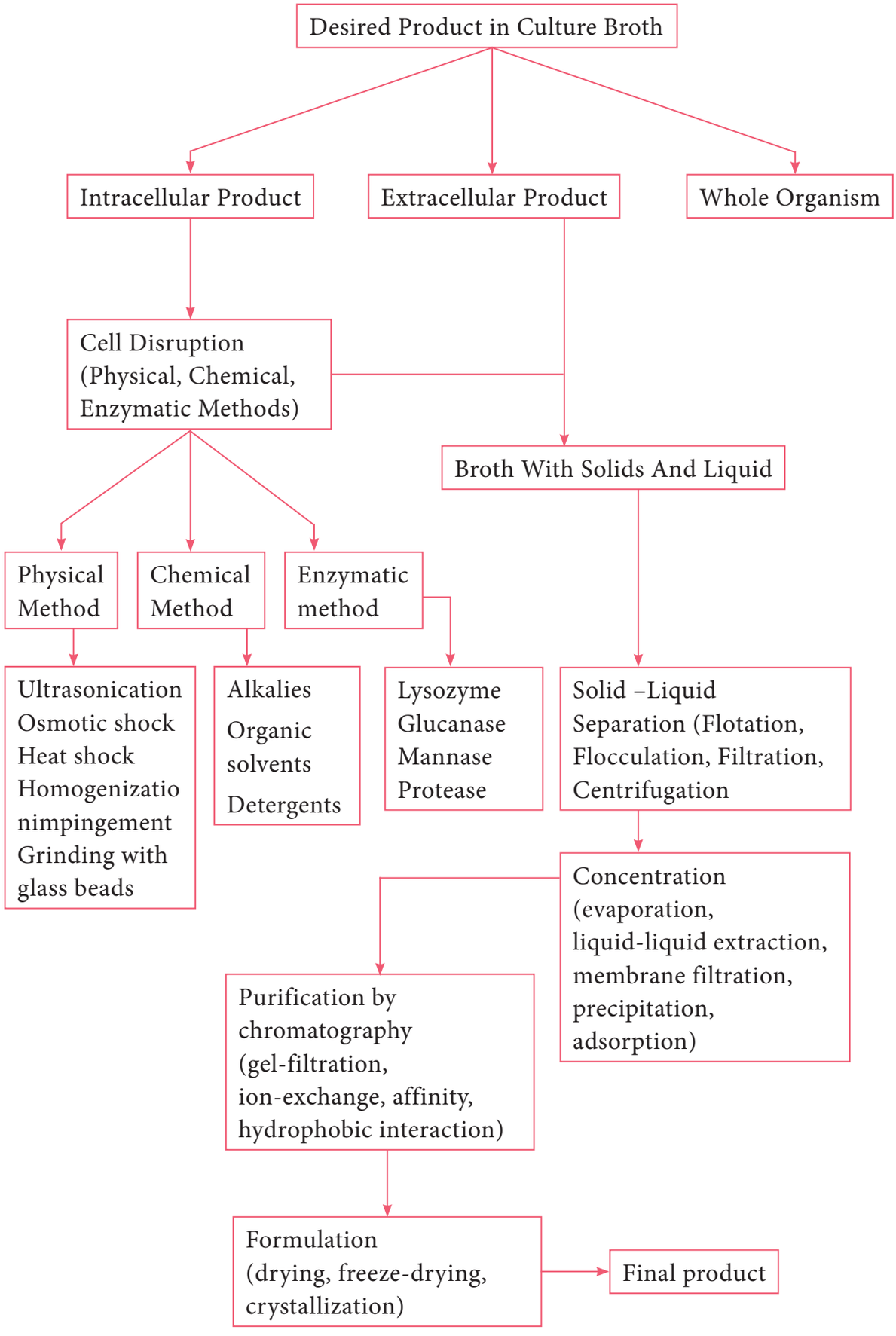
Followed by the fermentation, production, products are harvested or separated by downstream processing.

6.5.5 Downstream Processing

The various processes used for the actual recovery of useful products from fermentation or any other industrial processes are called downstream processing. The cost of downstream processing (DSP) is often more than 50% of the manufacturing cost, and there is product loss at each step of DSP. Therefore, the DSP should be efficient, involve as few steps as possible and be cost-effective. Methods involved in the downstream processing are outlined in the flowchart (6.2). Table 6.4 shows Difference between upstream and downstream processing.

Table 6.4: Difference between upstream (usp) and downstream (dsp) processing

USP	DSP
USP overall makes the procurement and maintenance of inoculum	DSP depends upon selection of cost–effective media
USP involves in strain improvement to enhance and yield	DSP concentrates on media optimization for maximum productivity yield and profit
It is a continuous development of selected strains to increase the economic yield	For DSP, fermentation conditions are optimized for the growth of micro organism or the production of a desired product



Flowchart 6.2: Downstream processing methods

6.6 Penicillin Production

Penicillin is a broad spectrum antibiotic. Penicillin is first obtained from the mould, *Penicillium notatum* (Figure 6.8).

Figure 6.8: Structure of *Penicillium notatum*

Penicillium chrysogenum is a high yielding strain, used for the commercial production of penicillin. This strain is highly unstable, so the spore suspensions are maintained in a dormant state to prevent contamination. Most penicillin form filamentous broth and hence is difficult to mix and it hinders oxygen transfer due to their high viscosity. This is avoided by using bubble columns air lift reactors which agitates the medium providing even oxygen distribution.

Penicillin has a basic structure 6-amino penicillanic acid 6-(APA). It consists of a thiazolidine ring with a condensed β-lactum ring. It carries a variable side chain in position 6. Natural penicillins are produced in a fermentation process without adding any side chain precursors. If a side chain precursor is added to the broth, desired penicillin is produced and it is called bio-synthetic penicillin.

Semi synthetic penicillin is one in which, both fermentation and chemical approach are used to produce useful pencillins. It can be taken orally and active against gram negative bacteria. (eg) Amphicilin. Nowadays, semi synthetic pencillins makeup the bulk of the penicillin market.

Infobits

In later (1939) using (Flemings' work) Howard Florey and Ernst Chain managed to purify penicillin in a powdered form. In 1941, they successfully treated a human. In 1943, they produced penicillin on a large scale. This helped immensely to treat causalities during the 2nd World War ww1 that had bacterial inflations due to their wounds.

The main objective of producing semi synthetic penicillin is to generate compounds with improved properties. (eg) acid stability, Resistance to enzymic degradation, broader spectrum of activity. Here side chain is removed to form (6- APA) via immobilization in a column of penicillin acylase. Penicilln G is converted to (6-APA) and phenyl acetic. Then it is chemically to acylated produce Semi Synthetic Pencillin.

New kinds of synthetic penicillin can also be produced which are readily absorbed by the intestine compared to natural penicillin. Example: Phenithicilin.

The initial strain of Penicillium chrysogenum (NRRL, 1951) was low yielding strain and so it is was treated with mutagenic agents such as X-rays, UV right and some other repeated methods to get a high yielding strain Q-176.

Production methods

Penicillin production is done by one of the following.

1. Surface culture
2. Submerged fermentation process

Inoculum Production

Inoculation methods

To inoculate fermentation medium one of the following methods can be employed.

1. Using dry spores to seed the fermentation medium.
2. Making suspension along with non toxic wetting agent like Sodium lauryl sulphate and inoculating germinated organism
3. Using pellet inocula obtained by the germination of spores

The lyophilized spores (or) spores in well sporulated frozen agar slant are suspended in water or in a dilute solution of a nontoxic wetting agent.

(1:10,000 sodium lauryl sulphonate)

↓

Spores are then added to a bottles containing wheat bran solution
It is incubated for 5-7 days at 24°C for heavy sporulation.

↓

The resulting spores are then transferred to production tank

↓

The micro organism in the inoculum tank is checked for contamination.

Production process

The production tanks are inoculated with a mycelial growth.

↓

Production medium contains following medium components.

Carbon source as Lactose, Nitrogen source as Ammonium sulphate, Acetate or Lactate (Corn steep liquor is the cheap and easy source of nitrogen)

Mineral sources as K, P (Potassium di hydrogen phosphate), Mg, S (Magnesium sulphate), Zn, Cu(Copper sulphate) (Corn steep liquor supply some of these minerals)

Precursor (Example: phenyl acetic acid) is added to the medium

↓

Antifoam agent (Example: corn or soybean oil) is added before sterilization

The sufficient aeration and agitation is given and are incubated at 25°C to 26°C for 3 to 5 days at PH range of 7 to 7.5

Penicillin Production

Process of penicillin production occurs in three phases:

First phase: Growth of mycelium occurs in this phase where the yield of antibiotic is low. The pH increases due to the release of NH_3.

Second phase: In this phase, intense synthesis of penicillin occurs due to rapid consumption of Lactose and Ammonium nitrogen. The mycelial mass increases and the pH remain unchanged (Figure 6.10).

Third phase: In this phase, the concentration of antibiotics decreases in the medium. Autolysis of mycelium starts, liberating Ammonia leading to slight rise in pH.

Recovery

After penicillin fermentation, the broth is filtered on rotary vacuum filter

↓

Mycelium is separated

↓

To the broth sulphuric acid or phosphoric acid is added

↓

Penicillin is converted into anionic form

↓

It is extracted in counter current solvent extractor, by using organic solvent, amyl acetate, methyl isobutyl (ketone)

↓

It is then back extracted with water from the organic solvent by adding potassium or sodium hydroxide

↓

Shifts between water and solvent aid in the purification of penicillin

↓

The resulting sodium or potassium penicillin is then crystallized

↓

Then it is washed, dried and used for commercial purpose

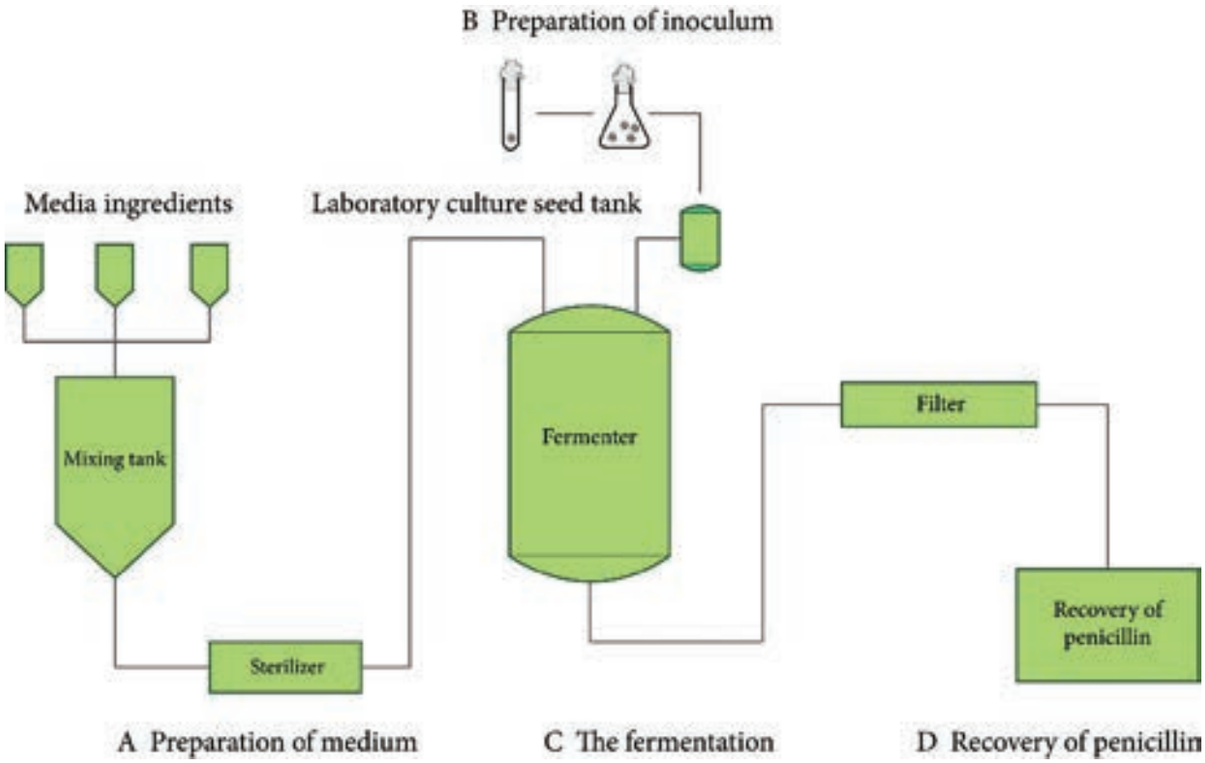


Figure 6.9: Production of Penicillin

6.7 Industrial Production of Wine

An alcoholic distilled beverage is produced by concentrating alcohol from fermentation by distillation. Beer or ale is produced by the fermentation of malted grains. Wine is prepared from grapes belonging to species *Vitis vinefera*. It is also produced from other fruits like peach, pear, dandelion and honey. Generally wine contains 16% of alcohol. Wine production from crushed grapes is called enology. The various forms of wine are listed below in the table 6.5.

Red wine is extracted from the skin of red grapes containing red pigment (anthocyanin). During the preparation of red wine, all the anthocyanin pigments are solubilized by the extract. Pink wine

is obtained from either pink grapes or red grapes in which fermentation last for only 12 to 36 hour and only less amount of anthocyanin pigments are solubilized. White wine is prepared from the white grapes or from the red grapes in which pigment involved in colouring is removed.

Generally yeasts are the natural microbiota of grapes

Both wild yeast and cultivated yeast are involved in the wine fermentation. Natural yeast is not potable because they do not produce much wine and are less alcohol tolerant and produce undesirable compounds, affecting the quality of the wine.

The cultivated wine yeast, *Saccharomyces ellipsoideus*, is used for commercial production. Figure 6.10 shows steps involved in wine production

Table 6.5: Shows different varieties of wine

Red wine	It has red pigments
White wine	It does not contain red pigments
Rose wine	It has less red pigments
Dry wine	It has more alcohol content
Sweet wine	It has more sugar content
Fortified wine	It is fortified with other alcoholic beverage
Sparkling wine	It has considerable amount of CO_2
Still wine	It does not contain carbon dioxide
Distilled wine	Brandy (alcohol content 21%)
Table wine	It has low alcohol and sugar content

Aqu-aori is the concept that oceans and other bodies of water, might impart unique characteristics on the aging process of submerged wine in water. The ocean provides a unique environment with cold temperatures, constant pressure, and little to no light and constant motion.

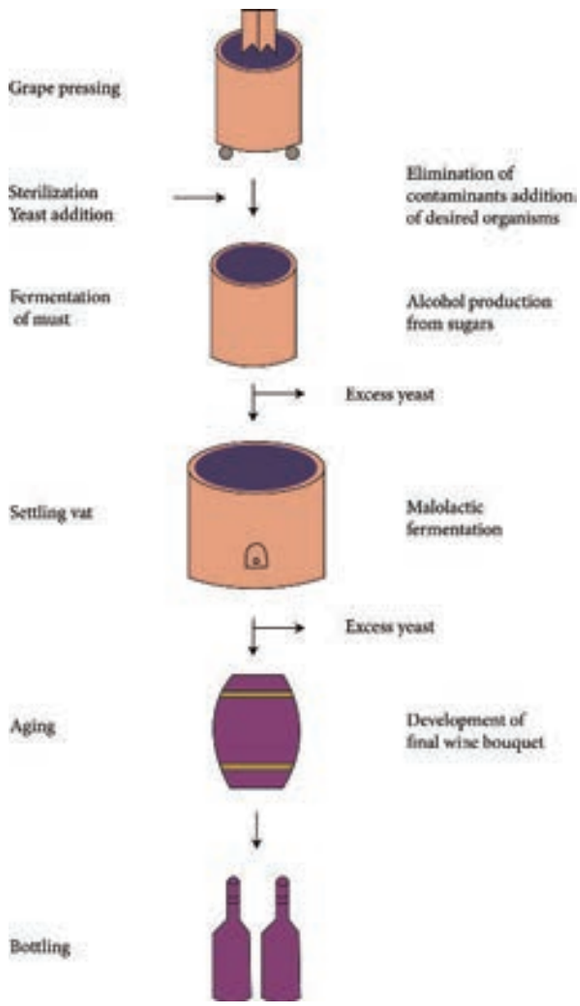


Figure 6.10: Steps involved in wine production

Steps involved in Wine production

Grapes are stemmed, cleaned and crushed

↓

Sodium or Potassium Meta – bisulphate is added to check the undesirable microorganism

↓

Must (crushed grapes) is treated with Sulfur dioxide to kill the wild yeasts and bacteria or sometimes pasteurized to destroy the natural microbiota

↓

Must is inoculated with *Saccharomyces ellipsoideus* (2.5%) and selected fermentation is carried from 50 to 50000 gallons at 20 to 24°C

↓

Oak, cement, stone glass lined metal are used as fermentor

↓

Temperature and time required for fermentation White wine: 10–21°C, 7–12 days; Red wine: 24–27°C, 3–5 days

↓

In red wine production, after three to five days of fermentation, sufficient tanin and colour is extracted from the pomace and the wine is drawn off for further fermentation

↓

Racking improves flavour and aroma, where wine is separated from the sediment containing yeast cells as precipitate form

↓

The wine is subjected to aging at lower temperature. Ageing process is typically much longer for red wine than white wine

↓

Wines are clarified in a process called fining. Fining is done by filtration through casein, tannin, diatomaceous earth or bentonite clay, asbestos, membrane filters or centrifugation

↓

The wine produced is placed in casks, tank and bottles

Infobits

Saccharomyces is called Brewing Yeasts, or Baker's Yeast. The brewing strains can be classified into two groups. The ale strains (*Saccharomyces cerevisiae*) and the lager strains (*Saccharomyces pastorianus* or *Saccharomyces carlsbergenris*). The ale strains are top fermenting strains. Lager strains are hybrid strains of *Saccharomyces cerevisiae* and *Saccharomyces eubayanus* and are often referred to as bottom fermenting. (*Saccharomyces* yeasts can form symbiotic matrices with bacteria and are used to produce Kombucha, Kefir, and Ginger beer).

After wine production, cork should be used for preventing the entry of air into the bottles. The presence of air allows the growth of vinegar bacteria that convert the ethanol to acetic acid. The final alcohol content of wine varies depending upon the sugar content of the grapes, length of the fermentation and type of strain used.

6.8 Industrial Production of Single Cell Protein

Single cell protein refers to the microbial cells or total protein extracted from pure microbial cell culture (monoculture) which can be used as protein supplement for humans or animals. During ancient times, the tribes in the Central African Republic used a spiral shaped Cyanobacterium named *Spirulina platensis* as food. They collected it as mats from the bottom of seasonally dried up ponds and shallow waters around Lake Chad and dried them in the sun and made small cakes called "Dihe".

Infobits

Zymology:

Zymology also known as "Zymurgy" (from the Greek–means, the workings of fermentation) is an applied science which studies the biochemical process of fermentation and its practical uses. It includes selection of fermenting yeast and bacteria, species and their use in brewing, wine making, fermenting milk and other fermented foods. The wine yeast Zymurgist one who studies or practices zymurgy; a knowledgeable brewer.

During the World war II, when there were shortage in proteins and vitamins in the diet, the Germans produced yeasts and a mould named *Geotrichum candidum* was used as food.

The term Single Cell Protein was coined by C.L Wilson (1966) at Massachusetts Institute of Technology (MIT), to represent the cells of algae, bacteria, yeasts and fungi, grown for their protein contents. The name was introduced by Prof. Scrimshow of MIT in 1967. The organisms like *Pseudomonas facilis*, P. *flava, Chlorella, Anabaena, Spirulina, Chlamydomonas*, and *Agaricus* are commonly used for SCP production. Large scale production of SCP is shown in the Figure 6.11

There are several methods available for SCP production. In the Japanese method, flat tray is used with artificial sunlight algae are cultivated in shallow ponds with mechanical stirrers or in deeper ponds (not more than 20–30 cm deep) with circulation pumps. Optimum, light is an important parameter for maximum growth of SCP. *Scenedesmus* sp. grows 20 times faster in optimum light than in natural conditions. Optimum temperature and optimum pH is varied according to the strain and intensity of light. Example: *Spirulina* is cultivated at 25–35°C with pH 9.5. Table 6.6 shows different types of microorganisms and substrates used for SCP production.

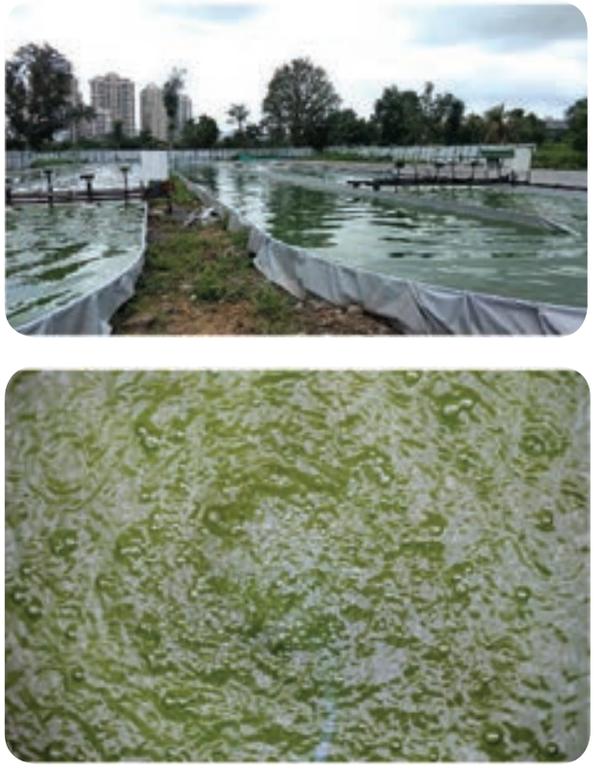


Figure 6.11: Large scale production of SCP

Steps involved in SCP production

Provision of carbon source with added nitrogen, CO_2, ammonia, trace minerals for growth

↓

Prevention of contamination by using sterilized medium and fermentation equipments

↓

Selected microorganism is inoculated in a pure form

Infobits

Some Commercial Products of Yeast

Sl. No.	Product	Micro Organism	Uses
1.	Baker's yeast, beer, wine, ale, bread	*Saccharomyces cerevisiae*	Baking industry brewing industry
2.	Soy sauce	*Saccharomyces rouxii*	Food Condiment
3.	Sour French bread	*Candida milleri*	Baking
4.	Commercial alcohol (ethanol)	*Saccharomyces cerevisiae* *Kluyveromyces fragilis*	Fuel, Solvent
5.	Riboflavin	*Eremotherium ashbyi*	Vitamin supplement
6.	Microbial protein	*Candida utilis* *Saccharomyces lipolytica*	Microbial protein from petroleum products. Animal food supplement (single cell protein) from paper-pulp waste

↓

Adequate aeration and cooling is provided

↓

Microbial biomass is harvested and recovered by flocculation or centrifugation flocculants

↓

Harvested algae are dewatered and dried on open sand beds

↓

Processing biomass and enhancing it for use and storage

Advantages of using microorganisms for SCP production:

1. Microorganisms grow at a very rapid rate under optimal culture conditions.
2. The quality and quantity of protein content in microorganisms is better compared to higher plants and animals.

Pruteen was the 1st commercial SCP used as animal feed additive with 72% of protein. Pruteen was produced from bacteria named *Methylophilus mehylotrophus* cultured on methanol.

In India, National Botanical Research Institute (NBRI) and the Central Food Technological Research Institute (CFTRI) are involved in the production of SCP.

In CPFTRI, SCP is produced from algae cultured on sewage.

3. A wide range of raw materials which are otherwise wasted, can be fruitfully used for SCP production
4. The culture conditions and the fermentation processes are very simple.
5. Microorganisms can be easily handled and subjected to genetic manipulations.

Table 6.6: List of microorganisms and substrates used for SCP production

Microorganisms	Substrates
Bacteria	
Pseudomonas sp.	Alkanes
Methylomonas sp.	Methanol
Yeast	
Candida utilis	Sulfite liquor
Lactobacillus bulgaricus	Whey
Fungi	
Aspergillus niger	Molasses
Trichoderma viridae	Straw, starch
Algae	
Spirulina maxima	Carbon di oxide
Scenedesmus acutus	Carbon di oxide
Actinomycetes	
Nocardia	Alkanes
Mushroom	
Agaricus bisporus	Compost, rice straw
Volvariella volvacea	Cotton straw

During the cultivation of SCP, care must be taken to prevent and control the contamination by other micro organisms, which produce mycoxins or cyanotoxins. This is controlled by using the fungus *Scytaliclium acidophilum* which grows at a low PH. It allows the hydrolysation of paper wastes to a sugar medium and also creates aseptic condition at low cost.

6.9 Industrial Production of Citric Acid

Citric acid is obtained from citrus fruits; pineapple etc., and after the development of microbial fermentation, citric acid production becomes very cheap, easy and cost effective. 70% of citric acid produced is used in food and beverage industry. Many microbial strains such as fungi *Aspergillus flavus, Aspergillus niger* and *Trichoderma viridae*, yeast *Hansenulla polymorpha* and *Candida lipolytica* are generally are involved in the production of citric acid.

Citric acid production can be carried out in the following three methods.

a. Koji process or solid state fermentation

b. Liquid surface culture

c. Submerged fermentation

Media used in citric acid production

Citric acid production is carried out by using carbohydrates and n–alkenes. Generally beet molasses, cane molasses, sucrose, commercial glucose and starch hydrolysate are used as carbohydrate sources. The carbohydrate material is diluted and mixed with a nitrogen source (ammonium salts or urea) and the pH and temperature are adjusted according to the process.

Inoculum development

Fungal strains that are used for production are stored in soil or silica gel in the form of spores. Spores are suspended in a freshly prepared sterile water containing Tween 80 and after a period of growth, it can be used as inoculum for large scale production.

Steps involved in citric acid production

Production Medium

Sucrose, beet molasses, used as carbon source need pretreatment, as it contains excessive amount of trace metals. So ferrocyanide or ferricyanide is added to the production medium before sterilization. Inorganic salts, carbon, hydrogen, oxygen trace metals. Nitrogen, potassium, phosphorus, sulphur and magnesium are taken in Aluminum or stainless steel shallow pans or tray (5–20 cm deep).

↓

Inoculated with spores of A. *niger* by blowing over the strains of *Aspergillus niger* for fermentation

↓

The medium is kept at 28–30°C with relative humidity 40–60% and aerated with purified air for 8–12 days

↓

Citric acid produced is determined by checking the pH or the total acid content of the broth.

↓

Fermented liquid is drained off and processed further for the recovery of citric acid

Infobits

Influence of trace metals in citric acid production:

Citric acid production is highly influenced by the trace metals. Particularly, iron and manganese in excess amount affect the citric acid production. They affect the cellular morphology and change pellets to filamentous growth (i.e.,) from productive form to unproductive form.

Recovery

The mycelial mat is pressed.

↓

Milk of lime (calcium carbonate) is added so calcium citrate is formed.

↓

Again sulphuric acid is added, so calcium sulphate is formed.

↓

The remaining citric acid solution is filtered and washed. Finally the impure solution of citric acid subjected to treatment with activated carbon and finally pure form of citric acid is collected.

Uses

It is used as a Acidulant in food, (Jams, Preserved fruits, Fruit drinks) and pharmaceutical industries.

1. It is mainly used in food and beverage industry (Jams, preserved fruits, fruit drinks)
2. It is used is pharmaceuticals, and other industrial processes
3. Citrate and citrate esters are used as plasticizers
4. It is used as a chelating and sequestering agent (Tanning of animal skins)

Generally citric acid obtained from citrus fruits, pineapple etc., After the development of microbial fermentation, citric acid production becomes very cheap and easy cost effective.

6.10 Immobilization

It is technique used for the physical or chemical fixation of plant, animal cells, organelles, enzymes or other proteins (monoclonal antibodies) onto a solid matrix or retained by a membrane, in order to increase their stability and make possible their repeated or continued use.

The immobilized enzyme is defined as the enzyme physically confined or localized in a certain defined region of space with retention of its catalytic activity which can be used repeatedly and continuously.

The selection of appropriate carrier and immobilization procedure is very essential procedure is very essential for the immobilization technique.

Various types of materials like cellulose, dextran, agarose, gelatin, albumin polystyrene, Calcium alginate polyacrylamide, collagen carrageenan and polyurethane, inorganic materials (brick, rand, glass, and ceramics, magnetic) are used for immobilization.

The linkage is mediated by ionic bonds, physical absorption or bio specific binding.

The immobilization methods can be classified into four categories

i. Carrier–binding

ii. Cross–linking

iii. Entrapping

iv. Combining

Among all these methods entrapping is discussed in brief.

Entrapping

The enzymes, cells are not directly attached to the support surface, but simply trapped inside the polymer matrix. Entrapping is carried out by mixing the biocatalyst into a monomer solution followed by a polymerization. It is done by change in temperature or by chemical reactions.

Advantages of immobilization

1. Immobilized growing cells serve as self proliferating and self regenerating bio catalyst
2. They are stable
3. They are used either repeatedly in a series of batch wise reactions or continuously in flow systems.

HOTS

The technique of immobilized enzymes may increase the use of enzymes in industry for product modification. Why? Give reason.

Summary

Industrial microbiology is a branch, of microbiology that deals with the study and uses of various microorganisms. The birth of industrial microbiology largely began with the studies of Pasteur on fermentation. Various products of both primary and secondary metabolites are produced by different microorganisms. Both primary and secondary screening is involved in the isolation of industrially important microorganisms. The isolated strains are modified for higher yield through various procedures (example) protoplast fusion. Thus strain improvement improves the fermentation efficiency. Fermentation is carried under a suitable parameters, in a controlled environment is called Fermentor. Fermentation process involves both cup stream processing and downstream processing. In cup stream processing, inoculum preparation scale up, preparation of medium and sterilization of media, are carried out.

Evaluation

Multiple choice questions

LS5C3

1. The term fermentation originates from a Latin verb _____

a. Wear

b. Fervere

c. Severe

d. Cheer

2. _____ metabolites are produced in small quantities during industrial production.

   a. Secondary metabolites

   b. Primary metabolites

   c. Tertiary metabolites

   d. Neutral metabolites

3. The microbes used in the industrial microbiology has these qualities

   Statement A: The strain should have stable biochemical and genetical characteristics.

   Statement B: It is a high yielding strain.

   a. Statement A alone is true

   b. Statement B alone is true

   c. Statement A and B are true

   d. Both A and B are false

4. Match the following:

A. Lactic acid	1. Penicillium chrysogenum
B. Citric acid	2. Lactobacillus delbrukeii
C. Penicillin	3. Saccharomyces
D. Ethanol	4. Aspergillus niger

   a. A4 B2 C1 D3
   b. A2 B4 C1 D3
   c. A1 B3 C4 D2
   d. A2 B4 C3 D1

5. _____ is an example for primary screening.

   a. Photography

   b. Cinematography

   c. Auxanography

   d. Telegraphy

6. Strain improvement is the technology of (Assertion) Manipulating and improving microbial strains. (Reason) It is done by Recombination and Protoplast fusion.

   a. Statement (A) is not supported by (R)

   b. Statement A is supported by (R)

   c. Statement (A) alone correct

   d. Statement (B) alone is correct

7. Match the following:

A. Penicillin	1. Aspergillus niger
B. Wine	2. Penicillium chrysogenum
C. Citric acid	3. Scenedesmus
D. Single cell protein	4. Saccharomyces cerevisiae

   a. A4 B1 C2 D3
   b. A3 B2 C1 D4
   c. A2 B4 C1 D3
   d. A2 B3 C4 D1

8. _____ are produced at the end of the growth phase or stationary phase.

   a. Tertiary b. Secondary

   c. Primary d. All the above

Answer the following

1. Define Fermentation?
2. What is bioreactor?
3. What is racking?
4. Define fining?
5. What are primary metabolites? Give one example?
6. What are secondary metabolites? Give example?
7. What are the characteristics of microbes in industrial microbiology?
8. Define primary screening with example.

9. Define secondary screening with example.
10. Crowded plate technique. Explain.
11. Giant colony technique–Explain in detail.
12. Write salient features of secondary screening.
13. What is strain development? What are the attributes of improved strains?
14. List any five methods of preservation of micro organisms.
15. Explain the components of fermentor.
16. What is cup stream processing?
17. Define downstream processing?
18. Difference between upstream and downstream processing.
19. Write the steps in downstream processing?
20. Write the components of fermentor and its function.
21. Explain the various types of fermentation median components used in Industrial microbiology?
22. Explain penicillin production any two process.
23. Define semi synthetic penicillin?
24. What are the different types of wine?
25. How will you prepare white wine from red grapes?
26. Explain the steps in wine production?
27. List the steps involved in SCP production.
28. What are the disadvantages of SCP?
29. Explain the steps involved in citric acid production.
30. What are the uses of citric acid.

Student Activity

1. Ask the students to prepare wine by using the grapes available in the super market.
2. Fermentor design and their components.
3. Wine production