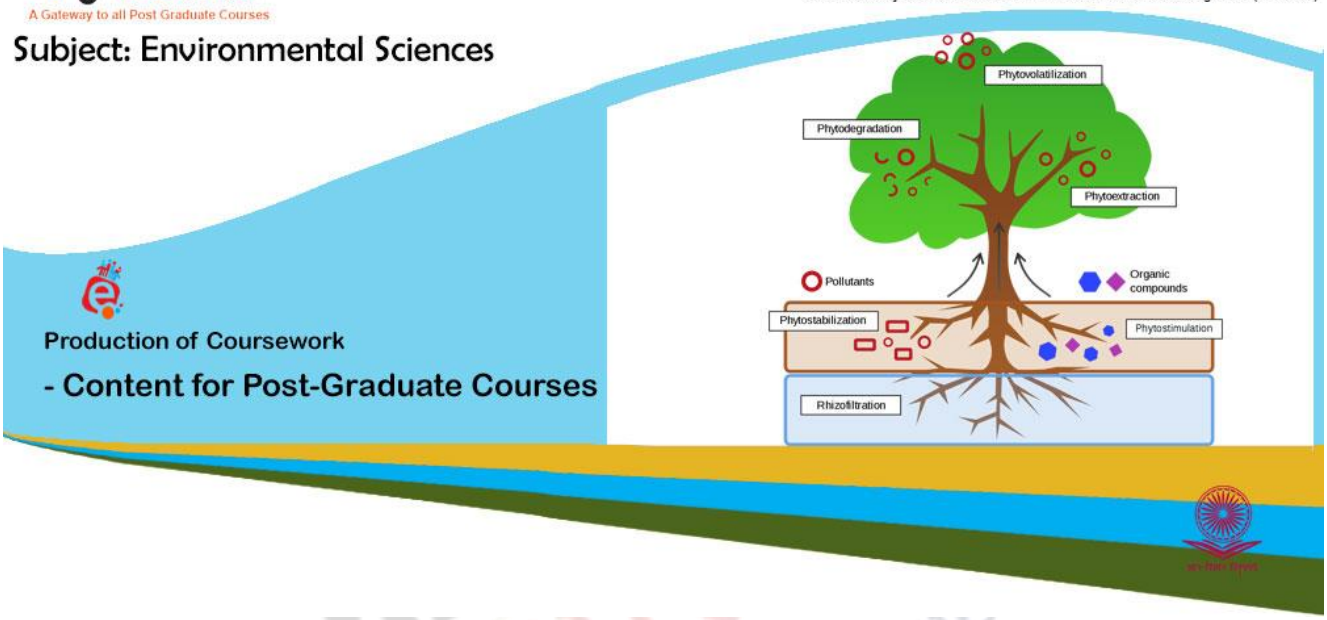

Paper No: 15 Environmental Microbiology & Biotechnology

Module: 21 Fermentation Technology

Development Team

Principal Investigator & Co- Principal Investigator	Prof. R.K. Kohli Prof. V.K. Garg & Prof. Ashok Dhawan Central University of Punjab, Bathinda
Paper Coordinator	Dr Babita Khosla Mahrashi Dayanand University, Rohtak
Content Writer	Dr. Hardeep Kaur Central University of Punjab, Bathinda
Content Reviewer	Dr Sunil Mittal Central University of Punjab, Bathinda
Anchor Institute	Central University of Punjab

Description of Module	
Subject Name	**Environmental Sciences**
Paper Name	Environmental Microbiology & Biotechnology
Module Name/Title	Fermentation Technology
Module Id	EVS/EP-XV/21
Pre-requisites	
Objectives	To study about Fermentation process, types of fermenters and their application
Keywords	Fermenter, Beer, Xanthan

Module 21: Fermentation Technology

1. **Introduction**
    - **1.1 Microbial Growth**
2. **Fermentation Process**
3. **Fermentation techniques**
4. **Application of Fermentation Technology**
    - **4.1 Beer production process**
    - **4.2 Production of Xanthan gum**
5. **Advantages of Fermentation**
6. **Disadvantages of fermentation**

1. Introduction

Fermentation word is derived from Latin verb "fervere" which means to boil. This technology has been perceived in different sense by the world of microbiologist and biochemist. For biochemists, "fermentation is a catabolic process leading to generation of energy". Whereas, according to industrial microbiologist, "fermentation is the process of mass cultivation of micro-organisms that convert substrates into valuable products through aerobic or anaerobic route". A general outline of the process is outlined in figure 1. Fermentation technology has wide application for the production of products such as organic solvents (acetone, alcohols), fermented beverages (wine, beer, whisky), and other products like enzymes, amino acids, vitamins, pharmaceuticals etc. The fermentation processes is dependent on microbial growth, which in turn is governed by many biochemical and physical parameters. The motive of all parameters is to provide the most suitable environment for the growth of micro-organisms. This chapter is dedicated to discuss the growth characteristics of micro-organisms, the types and needs of fermentation process and their applications.

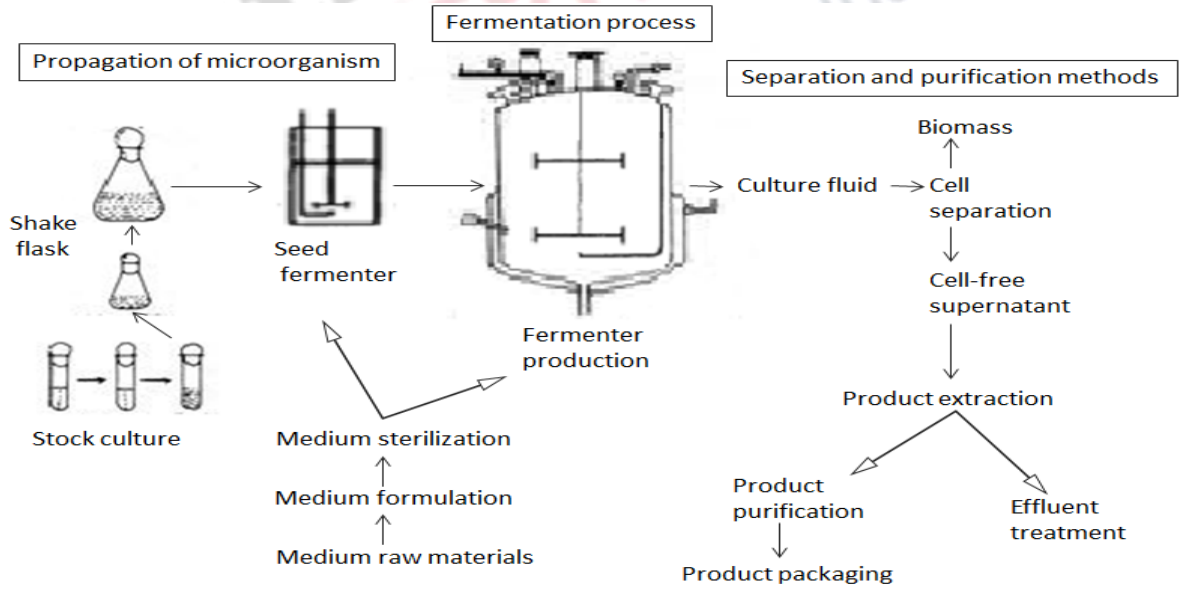

Figure 1: Outlines of Fermentation Technology

1.1 Microbial Growth

The most important criterion of a fermentation process is to achieve good yield of product which in turn is dependent on the proper microbial growth. The micro-organism require

optimum pH, temperature, oxygen, minerals, energy source and other raw material conditions to complete their life cycle under six phases as illustrated in figure 2 and discussed below.

a) **Lag phase** is the no microbial growth phase, also known as acclimatization period, when the newly inoculated micro-organism adapt to their new environment and hence show no increase in number.
b) **Acceleration phase:** is the period when microbes start increasing in number
c) **Log phase:** is the period when microbes demonstrated exponential increase in their number and utilizes most of the raw materials for their growth.
d) **Stationary phase** is the static stage when microbes does not show any change in their number and their growth arrest at this point, probably due to the depletion of energy sources in the media, constraints of space and accumulation of toxic end products.
e) **Death phase** is the time where microbes show steady decline in their number due to loss of ability to reproduce and indicate the climax of their growth period.

All these phases of microbial life illustrate a sigmoidal curve, and depending upon the type of product particular phase is considered for their harvest. For example, the processes where cell biomass is required, microbes are harvested after exponential growth. Whereas, when secondary metabolites are the major products, harvest is done after their production in stationary phase.

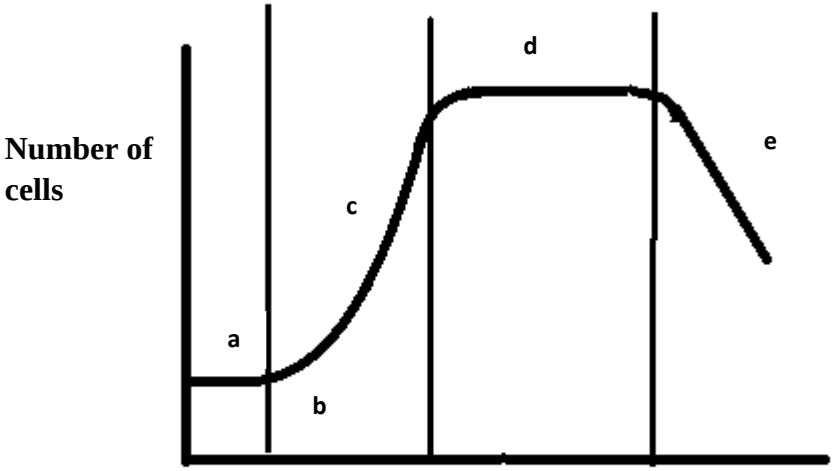

Figure 2: Growth phases of micro-organisms

The figure depicts the microbial growth in a batch fermentation system where one time addition of microbial culture and sterilized media components is done. It is a closed system, where no further addition or removal of materials are followed until the all the stages of microbial life cycle are completed and product is formed. After completion, the product is removed and processed under various downstream processes. Another type of fermentation process is fed-batch, where nutrients are fed more than one times during microbial cultivation, but products are harvested only at the end of the process. This method provides opportunity to control the yield and productivity of the process by adding limiting nutrients at defined phase of cell growth. For example the substrates such as ethanol, methanol or acetic acid may be added at the later stages of cell growth to avoid their inhibitory effect at initial growth phase. The most valuable method for high turnover of industrial products is the continuous fermentation that allows continuous supply of nutrients and raw materials. To maintain a static environment inside the vessel, products are also harvested continuously from the overflow of fermenter. The exponential growth of microbes is maintained for a prolonged period which is suitable for production of primary metabolites such as organic acids, amino acids, single cell protein etc.

2. Fermentation Process

Initially, Louis Pasteur described fermentation as “an anaerobic process carried out by yeast like organism to break complex sugars in simpler ones”. But, now fermentation includes anaerobic as well as aerobic processes that need maintenance of oxygen deficient or supplemented aseptic conditions. In case of anaerobic fermentation, no aeration device is needed as the gases generated during the process enable satisfactory mixing. The environment inside the vessel needs to be maintained oxygen scarce during the whole process which is maintained by flushing a mixture of CO_2, H_2 and N_2 in the head space of the fermenter. On the other hand, large volumes of oxygen approximately 60 times the medium volume are required for aerobic fermentation processes. Such fermenters need provision of efficient aeration and uniform mixing of fermenter contents.

To accomplish sterilized conditions along with uniform distribution of media, air, pressure and timely escape of products, a special vessel known as Fermenter is employed. In addition to above mentioned conditions, a fermenter should facilitate the following functions:

1. A fermenter should meet the requirement of containment that means to prevent the escape of viable cells during fermenter process or downstream processing.
2. It should provide continuous online monitoring of undergoing process through pH, temperature and pressure sensors.
3. A fermenter should provide stable environment required for the process with minimum consumption of operating power, maintenance labour, construction cost etc.
4. A pilot scale fermenter should provide the opportunity of scaling up the process.

A typical bioreactor has the following parts with specified functions as illustrated in figure 3. Principles of chemical engineering are applied to design a fermenter.

a. **Agitator** is used for uniform mixing of media constituents and to keep the cells suspension homogenous. Agitation also approves air dispersion, and random transfer of oxygen and heat. The most important function of an agitator is to reduce shear forces and forming produced during the biochemical conversion. To achieve homogenous environment through the vessel, different types of stirrer or impellers

such as disc turbine, vaned disc, open turbine and propeller are used. All these agitators are designed to achieve objectives of gas-phase and bulk fluid mixing.

b. **Aeration System** plays the crucial role along with agitators to supply sufficient oxygen to growing cells without causing any damage to them. The device used for this purpose is known as sparger. Sparger is needed for efficient aeration by bubbling of air through the medium, as surface aeration is inefficient for submerged cultures. The kind of aeration system required in a fermenter is characterized by the type of process and attributes of media constituents and microbial culture. Sometimes only aeration could achieve the functions of proper mixing and oxygen supply which eliminated the need of extra agitator. Such conditions are dictated by the less viscous broth, and bacterial cultures. For high viscosity broths, it is desired that Fermenters with height/diameter ration of 5:1 provide uniform mixing by aeration system only and save equipment and power cost. But, for fungal cultures proper mixing by agitators is required. The spargers have been employed with variable designs such as orifice sparger, nozzle sparger or combined sparger- agitator.

c. **Baffles** are the metal strips attached radially to the vessel wall to prevent whirl formation and to perform efficient aeration. Their length may be one tenth of the vessel diameter with diameter less than 3 meters and their number varies from 4 to 8. Wider baffles are advised to have positive effect on agitation efficiency as compared to narrow baffles. A gap between the baffles and fermenter wall prevent microbial growth at the vessel wall.

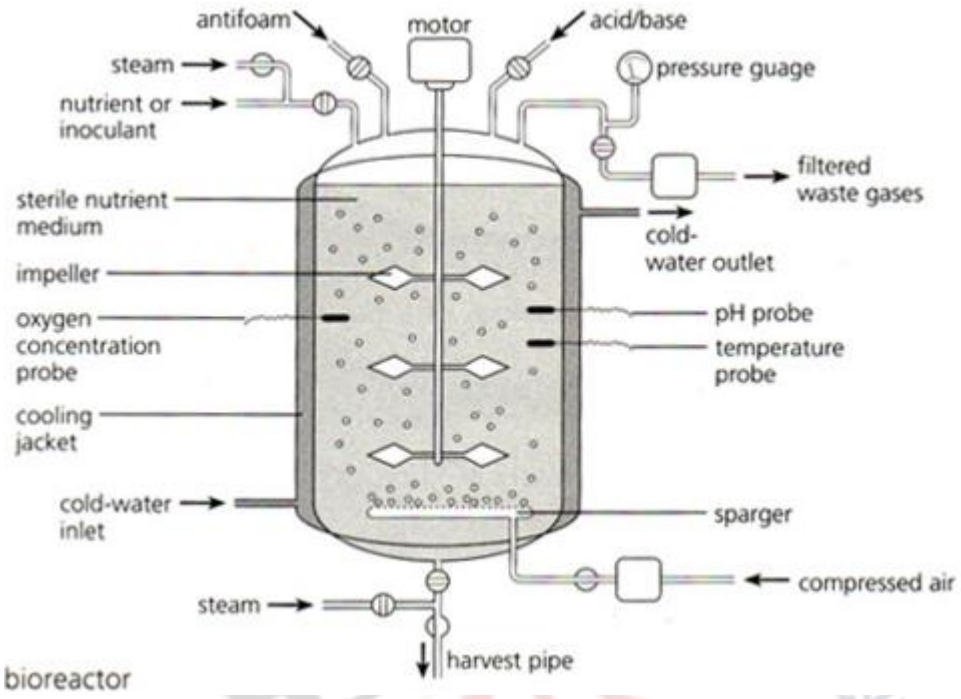

Figure 3: A typical design of a fermenter

http://2010.igem.org/Team:UCL_London/Fermenter_Mechanics

Specially designed fermenters are employed for specific product production which depends on the process kinetics, the kind of raw materials used and magnitude of process parameters required during the process. The most satisfactory designs have been discussed here.

1. **Stirred tank fermenters** are usually used for batch fermentation and are equipped with impellers for proper stirring and sparger for efficient aeration. These are usually made up of glass or steel body of 20 l capacity. The height to diameter ratio (aspect ratio) of a stirred tank bioreactor is usually between 3 to 5. However, it may be reduced to 2 for animal cell culture applications. The diameter of the impellers are typically 1/3 rd of the vessel diameter. The negative pressure produced by the rotation of impellers allows efficient gas transfer and circulation without causing any damage to culture. Different types of impellers such as Rustom disc, concave bladed, marine propeller etc. are used dependent upon the product and environmental conditions required during the process. In a stirred tank fermenter steady state conditions are achieved by either chemostatic or turbido static operation. The former includes alteration of the flow rate of the vessel to a suitable value that allows the micro-

organisms, substrates and biochemical product concentration to attain their natural levels. The turbidostat requires an experimental determination of the turbidity (ie, indirect measurement of microbial concentration). Once the culture attains the predefined density, a fixed volume of culture is withdrawn and same volume of fresh medium is added. Stirred tank fermenters provide the advantages of continuous operation, low operation cost and control over the process.

2. **Tower fermenter/ Bubble Column** are named so because of their elongated body with aspect ratio of 10:1 and introduction of air at the base from perforated pipes. The bottom aeration creates a high pressure zone which facilitates oxygen solubility in the medium. The expanded head space reduces pressure and allows expulsion of gases produced during the biochemical reaction. Such fermenters utilize non-agitated vessels for mycelial fermentations of products such as citric acid and tetracycline.
3. **Cylindro-conical fermenters** are used mostly in brewery industry. Such fermenters consist of a hemispherical top and a conical base for easy separation of yeast or fermenting microbe. The usual aspect ratio is 3:1.The mixing is achieved non-mechanically by rapid rising of CO_2 bubbles in the vessel. Owing to their use for beer production, they are fitted with cooling jackets that are used for flocculation and settling of yeast. Cylindro-conical fermenters provide advantages of reduced process and maturation time and possibility of primary fermentation and conditioning in the same vessel.
4. **Air-lift fermenters** are used for aerobic fermentations that ensure cyclic liquid flow for efficient aeration. The fermenter is divided into a riser tube and a down comer tube. Oxygen is introduced through the base of the riser tube by a sparger that allows rise of broth towards upward. The down tube is less aerated due to which the density of the broth increases and causes its downward movement. This cyclic process continuous and the undesired gases are blown out of the vessel through gas disengagement chamber. Air-lift fermenters may be of inner loop or externally placed riser tube (figure 4). In both the cases, liquid density difference between the riser tube and down comer tube drives the circulation of the medium. Such fermenters are employed for the production of methanol, single cell protein or waste water treatment

where high rate to aeration and agitations are required for the process.The external loop riser configuration is flexible and the change in the conformation increases the oxygen transfer rate as well as mass transfer coefficient for a specific airflow rate. Such reactors reduce the operational cost for impelling air through the fermenter and thus are preferred over stirred tank fermenters.

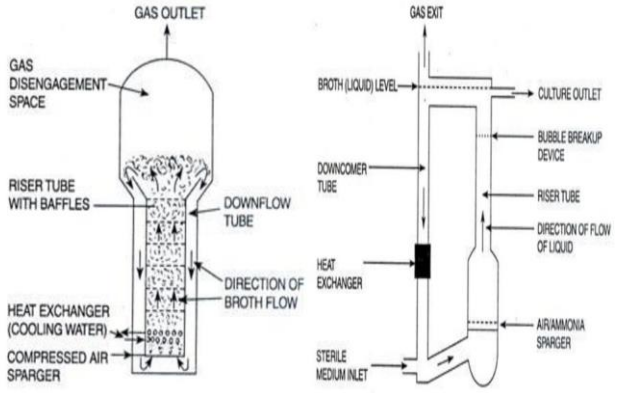

Figure 4: Air lift fermenter with inner and external loop configuration

(P.F. Stanbury and A. Whitaker, 1986)

5. **Fluidized Bed fermenters** are packed with solid material suspended in an upward flowing fluid. The solid material is usually a catalyst which is responsible for biochemical conversion of substrates in the medium into products. The velocity of liquid is maintained such that the solid material behaves like a fluid and surpasses any need of agitation. These reactors are basically used to produce petroleum products from crude oil, rubber, polyethylene etc. through some catalytic process. Fluidized bed reactors provide the advantage of even temperature gradient, uniform particle mixing and continuous operation ability. But, to keep the solid material in suspended form high pumping power is consumed and a large vessel size is needed that constraint its dynamic application.
6. **Photo-bioreactors** are used for the systems that need proper illumination through sunlight or artificial light. These have large surface to volume ratio consisting of flat panels or array tubes arranged vertically or horizontally to facilitate better light

penetration and gas transfer (figure 5). The culture which is generally algae or cyanobacterium are circulated through the transparent glass or plastic tubes using airlift pumps and never allowed to sediment. A cooling system is always required in such fermenters to avoid temperature rise, which is maintained at 25 to 40 ºC. The microbes grow during day light and yield product at night hours.Various configuration of photo-bioreactors are possible such as tubular, horizontal, Christmas tree etc. which provide the advantage of space saving and protection from contamination.

Figure 5: A tubular Photo-bioreactor

(https://en.wikipedia.org/wiki/Photobioreactor)

3. **Fermentation Techniques**

Solid State Fermentation	Submerged Fermentation
Microorganisms are cultivated on the surface of a liquid or solid substrate.	Microorganisms grow in a liquid medium.
Complicated and rarely used in industry.	Simple and used in routine.
Used in Production of Mushroom, Bread, Cocoa and temph etc.	Protein, Biomass, antibiotics, enzymes and sewage treatment are carried out by submerged fermentation.

4. Application of Fermentation Technology

Advances in fermenter designing and fermentation technology have led to the commercialization of a number of fermented products. Various kinds of food and food

additives are manufactured using industrial fermentation technology in developing countries. Wild type and recombinant microorganisms are used in the production of following products.

1.	Alcoholic beverages	Whisky, rum, brandy, Beer and wine
2.	Milk and Milk Products	Cultured milks, yoghurt, cheese
3.	Microbial flavors	Vanillin, benzaldehyde and lactones
4.	Biofuels	Ethanol, acetone-butanol
5.	Microbial polysaccharides	Dextran, xanthan gum and pullulan
6.	Food additives and ingredients	L-glutamic acid and L-aspartic acid
7.	Vitamins	Vitamin A, C, B_{12}, and riboflavin
8.	Enzyme	Amylase, invertase, Protease
9.	Organic acid	Lactic acid, citric acid, acetic acid

The end product of a fermentation process depends upon the kind of microbe used for the process. Many bacteria, protists, fungi and animal cells produce lactic acid, water and carbon dioxide, whereas yeast and plant cells yield alcohol, water and carbon dioxide. Thus, most of the alcoholic beverages are produced by the action of yeast on carbon sources such as malted barley, millet, sorghum, cassava etc. Among all the fermented alcoholic beverages, beer making (brewing) is the oldest and the most researched process. So, the next section would be dedicated to discuss brewing process.

4.1 Beer production process

Brewing is basically a two phase process, where in first phase a carbon source is soaked in water to extract its flavours (steeping) and then fermentation of the sweet liquid is carried out by yeast. The most preferred carbon source for brewing is malted barley because of its fibrous husk and rich content of amylase that converts starch into sugars. But, certain secondary carbon sources such as maize, rice or sugar may be added as adjuncts to provide specific characteristics to the final product and to make the process cost effective. The brewing process takes place under steps that includes malting, mashing, lautering, boiling, fermentation, filtration, packaging (figure 6).

The malting process includes soaking of the grain in water to allow germination (for about 5 days) and then roasting (kilning) the partially germinated grain at different temperature and

times to produce different colours of malt from the same gain. This also results in development of different flavours of the beer. There are basically three varieties of malt based on their kilning temperature:

- Pale malt is dried by roasting at maximum temperature of 85 ºC.
- Caramel malt is roasted at 150 ºC.
- Roasted malt is heated upto 225 ºC that imparts a dark colour and characteristic flavour to the malt.

Kilning is followed by milling that breaks the kernels and exposes the part that contains most of the carbohydrates and sugars.

Mashing includes conversion of starch into sugars by the integral hydrolysing enzymes of the malt. This step takes place in large vessels known as mash tun that allow mixing of milled grain with hot water which is followed by multistage heating. The four major stages of mashing are:

- Peptonizing rest: takes place at temperatures around 50 ºC. At this stage the proteins are broken down into amino acids which are utilized by the yeast for their growth. In addition the substances that forms foam in beer are produced during this stage.
- Maltose break (62 – 64 ºC): marks the degradation of starch stored in the grains into simpler sugars such as maltose, glucose etc.
- Saccharification rest (70 – 72 ºC): is the complete convertion of water dissolved starch into sugars which is necessary to produce clear beer without any turbidity due to undigested starch.
- Mash out (up to 80 ºC): is done at high temperatures to stop the activities of the enzymes, to prevent excessive degradation of substances and production of undesirable flavours. This step is also used to reduce mash viscosity and to free out more sugars from the grains by sprinkling water over them (sparging).

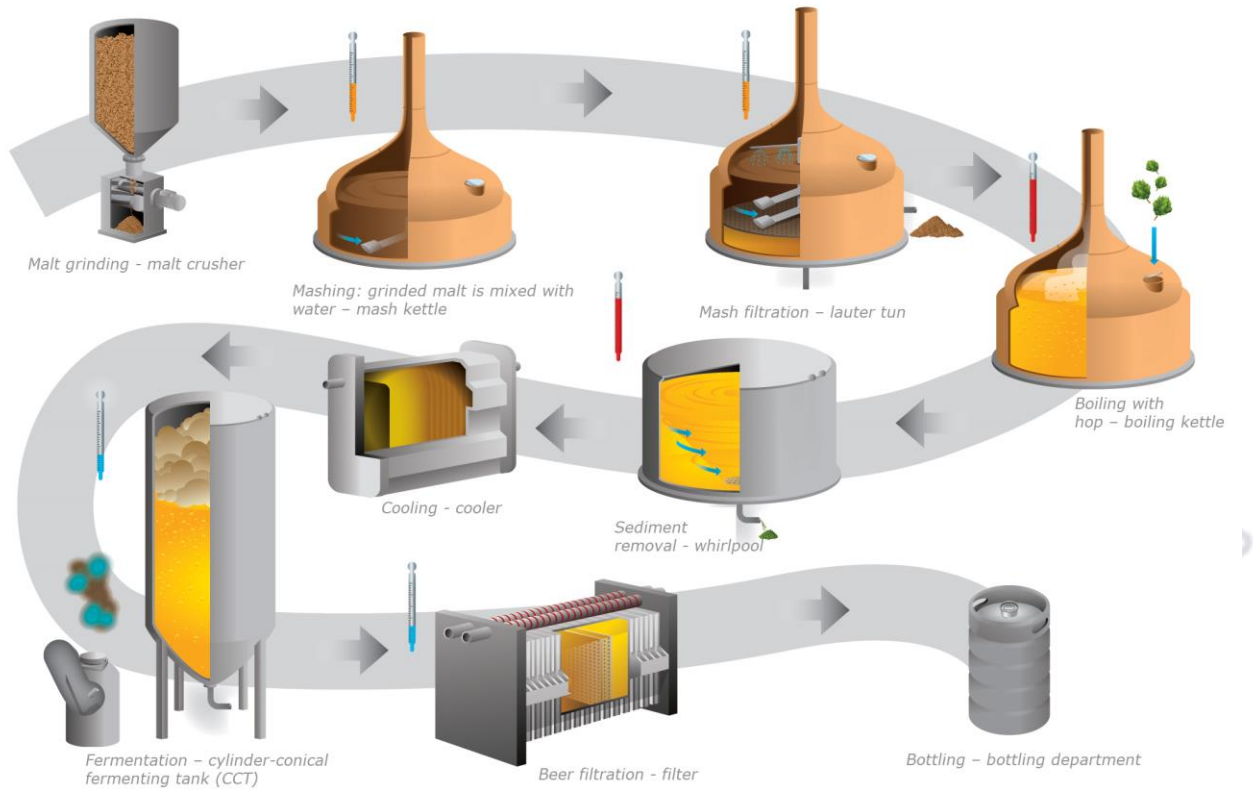

Figure 6: Beer production process

(http://eng.baltika.ru/m/6320)

Mashing may be done by two methods, infusion or decoction. Infusion includes heating of grains in one vessel, whereas decoction comprises boiling of a proportion of grains which are then returned to the mash to raise its temperature. The mashing process results in sugar rich liquid called “wort”, which is separated from the solid residues by straining and the process is called lautering. Wort is transferred to a copper vessel, and boiled with a flavouring agent “hop” which enhances aroma and bitterness of the beer. This stage is very important as the nature and quantity of hops decides the flavour and other characteristics of beer. Boiling in traditional copper vessels provides protein precipitation, enzyme activity termination, concentration and sterilization of the wort. After boiling the solid particles are separated in a settling tank known as “whirlpool”. In a whirlpool the centrifugal force separates out the heavy solid residues at the centre of the bottom of the tank.

The next step in brewing is fermentation, but before that the temperature of wort is lowered down to the temperatures optimal for yeast activity. Generally the wort is passed through a heat exchanger containing many ridged plates that help to dissipate heat faster. Fermentation is carried out by the yeast strain *Saccharomyces cerevisiae* (top-fermenting) or *Saccharomyces pastorianus* (bottom-fermenting). The terms top or bottom fermenting are no longer valid as cylindro-conical vessels are used generally for fermentation and conditioning purpose. The separation of yeast in such vessels is done from the bottom only in its flocculated form (figure 7)

Figure 7: Cylindro-conical vessels for beer fermentation

Fermentation is classified as warm, cool or spontaneous as per the temperature requirement.

- Warm fermentation is carried out by *Saccharomyces cerevisiae* at 15 – 20 ºC temperature.
- Cool fermentation is carried out by *Saccharomyces pastorianus* at around 10 ºC temperature.
- Spontaneous fermentation is carried out by the wild yeast and bacteria of the oak barrels in which the fermentation takes place. There is no deliberate addition of specific yeast strain, and a mixed culture is generally employed for the fermentation.

Certain brands of beer follow an ageing period of one to few months at freezing temperature, also called the conditioning or lagering, when the beer is separated from the bottom flocculated yeast and allowed to rest to develop a unique taste.

4.2 Production of Xanthan Gum

Another commercial product manufactured by microbial fermentation is xanthan gum, which is a natural polysaccharide used as thickening agent in food industries, stabilizing agent in pharmaceutical industries and as friction reducer in petroleum industries. The expected market of xanthan is about to US $400 million with a production of 80,000 tons/year in 2016. It was discovered in the 1950s at the Northern Regional Research Laboratories (NRRL) of the United States Department of Agriculture. Xanthan gum is a hetero polysaccharide. Its primary structure consisting of repeated pentasaccharide units formed by two glucose units, two mannose units, and one glucuronic acid unit, in the molar ratio 2.8:2.0:2.0. The negatively charged carboxyl groups on the side chains encourage the molecules to form very viscous fluids when mixed with water (figure 8). Xanthan is Soluble in hot or cold water and insoluble in most organic solvents. It has good stability when exposed to freeze/ thaw.

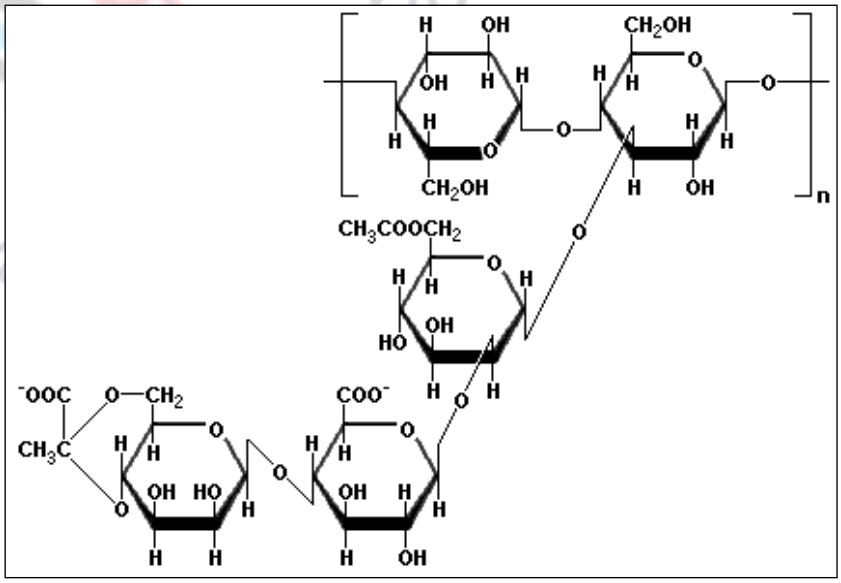

Figure 8: Structure of Xanthan

Microbial Production of Xanthan gum

Xanthan Gum is produced by the bacterium ***Xanthomonas campestris***, which is found on cruciferous vegetables such as cabbage and cauliflower.

Media preparation

- Inexpensive and complex media (tap water, glucose, sucrose, and starch)
- Carbon is primary and nitrogen as secondary limiting substrate

Fermentation

- Submerged, aerobic
- Xantham gum as secondary metabolite
- High viscosity as a potential problem

After fermentation HCl is added to the growth medium followed by small amount of alcohol. The growth medium is centrifuged to remove the bacterial cell. After that, more is added to precipitate xanthan gum. The precipitate is dissolved into water and precipitated with alcohol again to get food grade xanthan gum. Finally, the precipitate is dried and grounded into fine powder.

Key steps in typical production of xanthan

S. No.	Process step	Scale and operations	Supports
1	Culture preservation of ***X. campestris***	**Long-term** : lyophilized; frozen **Short-term** : solid media slants or plates	Strain improvement Test for culture viability
2	Inoculam build-up	Shake flasks; Inoculam fermenters	Growth medium composition; Controlled operational conditions; Test for contaminants
3	Production stage	Bioreactor	Equipment design; Production medium composition; fermentation conditions; controlled operational conditions

4	Harvest	Thermal, Chemical or enzymatic; Centrifugation or filtration	Process development of cell deactivation and removal
5	Isolation	Precipitation; filtration	Development of extraction and purification methods

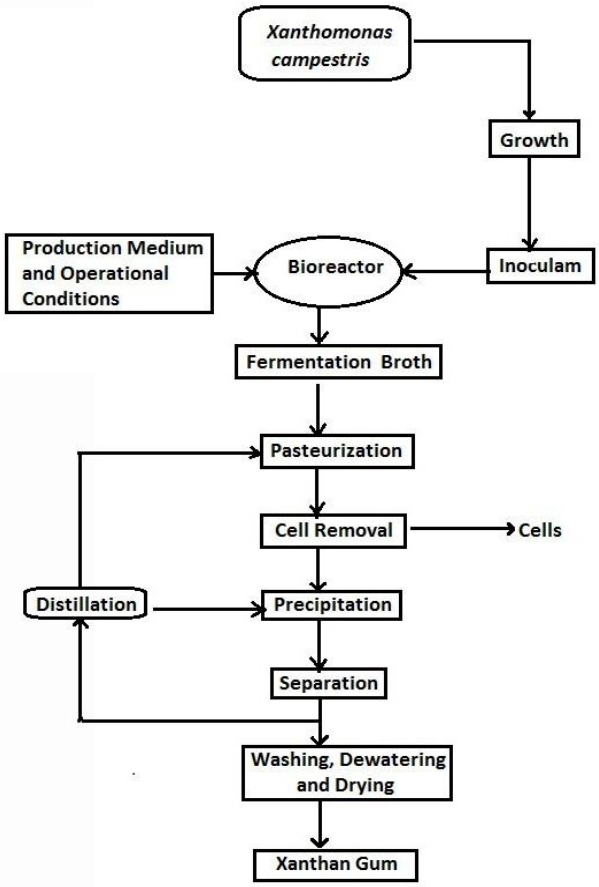

Figure 8: Process for making xanthan

Applications of Xanthan:

Applications	**Concentration (%w/w)**	**Functionality**
Frozen foods	0.05-0.2	Improves freeze- thaw stability

Syrups, toppings, relishes, sauces	0.05-0.2	Thickener, heat stability and uniform viscosity
Beverages (fruit and non-fat dry milk)	0.05-0.2	Stabilizer
Agriculture (additive in animal feed and pesticide formulations)	0.03-0.4	Suspension stabilizer; improved sprayability, reduced drift
Baked goods	0.1-0.4	Stabilizer; facilitates pumping
Enhanced oil recovery	0.05-0.2	Reduces water mobility by increasing viscosity and decreasing permeability
Textile printing and dyeing	0.2-0.5	Control of rheological properties of paste; preventing dye migration
Salad dressings	0.1-0.5	Emulsion stabilizer, suspending agent, dispersant
Dairy products	0.5-0.2	Stabilizer; viscosity control of mix
Pharmaceuticals (creams and suspensions)	0.1-1	Emulsion stabilizer; uniformity in dosage formulations
Cosmetics (denture cleaners, shampoos, lotions)	0.2-1	Thickener and stabilizer
Dry mixes	0.05-0.2	Eases dispersion in hot and cold water

5. Advantages of Fermentation Technology

1. Preservation and enriches food, improves digestibility, and enhances the taste and flavors of foods.
2. Potential of enhancing food safety by controlling the growth and multiplication of number of pathogens in foods.
3. Important contribution to human nutrition, particularly in developing countries, where economic problems pose a major barrier to ensuring food safety.
4. Low energy consumption due to the mild operating conditions relatively low capital and operating costs relative simple technologies.
5. They cause specific and controlled changes to foods by using enzymes.
6. Preservation and detoxification of the food.
7. Waste treatment.
8. Health related product.

6. Disadvantages of Fermentation

1. Hazardous contamination always exists in fermented food.
2. The uneven distribution of salt in lactic acid fermentation fish products and contamination of *Aspergillus flavus* in traditional starter culture for rice wine and soyabean sauce result in severe food poisoning incidences.
3. Health (obesity).