INDUSTRIAL MICROBIOLOGY

Dr. Ariyah Terasawat

Industrial microbiology:

- A branch of applied microbiology.
- It refers to the use of microorganisms in commercial enterprises.
- The microorganisms are processed under controlled temperature, pH to produce valuable products

HISTORY

As early as 6000 BC the Babylonian and Sumerians used yeast to make alcohol.

During first world war, a British scientist Chaim Weizmann developed fermenter for the production of acetone.

Pasteur developed a new way of approach to industrial microbiology.

Citric acid fermentation ranks in Pfizer's Brooklyn facility, circa 1920s.

DEVELOPMENTAL PHASE IN INDUSTRIAL MICROBIOLOGY

1.Relatively ancient, involves fermentation of alcoholic beverages, bread .

2.Large scale cultivation of yeast cell for brewing and baking industries.known as fermentation industry,vessels, medium were introduced.

3.Involves the batch culture of microbes under aerobic condition.pencillin production largely occur.

4.Use of microorganisms as a bulk sources of protein for animal and human food called single cell protein(scp). 5.Biotechnology, recombinant DNA technology is applied in microorganisms for developing useful products.

FERMENTATION

Chemical conversion of sugar to acid, ethanol, gases.

Fermentation are done in large vessel called Fermenter, microbes are grown under controlled temperature,PH.

Science of fermentation called zymology.

FERMENTATION STEPS

- 1. Microbial strain selection
- 2. Fermenter selection
- 3. Fermenter media selection
- 4. Fermenter process
 - **1. Upstream process**
 - 2. Downstream process
- 5. Quality control and assurance
- 6. Packaging

WHY MICROBES?

*Microbes are the mini chemical factory. *Posses a broad variety of enzymes.

*High metabolic activity,so it can grow and multiply rapidly.

*Possess a large surface area for the quick absorption of nutrients.

*Grow on inexpensive

substrate.Byproduct of reaction will be the substrate of another.

- Amenable to genetic manipulation.
- Should not be pathogenic to humans and animals.



© Encyclopædia Britannica, Inc.

Fermenter selection

DIFFERANCE BETWEEN SOLID STATE AND SUBMERGED FERMENTATION

SOLID STATE FERMENTATION

- MIXING AND DIFFUSION OF SUBSTRATE AND PRODUCT IN RELATION TO BIMASS IS MUCH SMALLER IN SSF.
- SOLUBILITY AND DIFFUSION OF OXYGEN AND OTHER NON-POLAR GASES IS GREATER IN SSF.
- HEAT CONDUCTION IS MUCH SMALLER IN SSF.
- WATER CONTENT IS SMALLER IN SSF.

SUBMERGED FERMENTATION

- MIXING AND DIFFUSION OF SUBSTRATE AND PRODUCT IN RELATION TO BIMASS IS MUCH HIGHER IN SSF.
- SOLUBILITY AND DIFFUSION OF OXYGEN AND OTHER NON-POLAR GASES IS SMALLER IN SSF.
- HEAT CONDUCTION IS MUCH HIGHER IN SSF.
- WATER CONTENT IS HIGHER IN SSF.

Fermenter selection

(a) The arrangement of moist solid particles and the continuous gas phase in SSF systems involving a filamentous fungus (left-hand side) and a unicellular organism (right-hand side).



(b) Other systems that involve growth on solids, but which are not defined as SSF due to the large amount of water in the inter-particle spaces. The left-hand diagram represents a trickling-filter type system while the righthand diagram represents a suspension or slurry system

Solid state fermentation (SSF)



Applications of SSF

- SSF technology has been used for many centuries. Some examples of traditional SSF processes are:
 - 1. Tempe involves the cultivation of the fungus *Rhizopus oligosporus* on cooked soybeans. The fungal mycelium binds the soybeans into a compact cake, which is then fried and eaten as a meat substitute. This fermented food is quite popular in Indonesia;
 - 2. The *koji* step of soy sauce manufacture, which involves the cultivation of the fungus *Aspergillus oryzae* on cooked soybeans. During the initial SSF process of 2 to 3 days, the fungal mycelium not only covers the beans but also secretes a mixture of enzymes into them. The fermented beans are then transferred into brine, in which, over a period of several months, the enzymes slowly degrade the soybeans, leaving a dark brown sauce.
 - 3. Ang-kak, or "red rice", which involves the cultivation of the fungus *Monascus purpureus* on cooked rice. The fungus produces a dark red pigment. At the end of the fermentation the red fermented rice is dried and ground, with the powder being used as a coloring agent in cooking.







Submerge fermentation (SMF)



COMPONENTS OF FERMENTER

- **1. Vessel: to carry out the process**
- 2. Impeller:proper mixing,heat transfer, prevents sedimentation.
- **3. Sparger:device that introduce air into medium.**
- Baffles:to prevent vortex and to improve aeration efficiency.
- 5. Temperature control
- 6. PH/control
- 7. Feed parts: to add nutrients



FERMENTATION MEDIA

- Carbon source: wheat,rice
- Nitrogen source: yeast extract





BATCH FERMENTATION

- Is a process which large volumes of nutrient medium is inoculated initially.
- About 2 or 4% microorganisms are added initially.
- After fermentation, microbes are removed and materials are isolated.
- Growth phases:
- 1. Lag phase(adopt to new environment)
- 2. Exponential phase(increase in number)
- 3. Stationery phase
- 4. Decline phase







CONTINUOUS FERMENTATION (Perfusion)

- The medium is added continuously to the tank to replace that which has been fermented at same rate.
- Chemostat: used to provide constant flow, keeping microorganisms in the logarithmic phase of growth.
- Turbidostat: instrument measure the turbidity of the microbial population to indicate the level of growth.

Remain in a logarithmic phase.

FED BATCH FERMENTATION

*substrate are added in increments as fermentation progress.

*Critical elements are added in small doses and it continued during production phase.





Pilot scale (50-2000L)



Industrial scale (2000 L >)



Laboratory scale (<50L)

Knowledge Rheology Mass transfer Thermodynamic Chemical kinetic Microbial kinetic Fermentation design



$$\frac{dP}{dt} = r_p = q_p X = (\alpha \mu + \beta) x$$

$$P = \text{Product concentration (} r_p = \text{Rate of productionn (gP}) \\ q_p = Y_{p/x} \mu = \text{specific product}$$

$$P = P_{p/x} \mu = P_{p/x} \mu$$

(gP/L) P/L/hr) ction rate (gP/gX/hr)





PRODUCTION OF ANTIBIOTICS

- The study of antibiotics began from, discovery of penicillin in 1929 by Alexander Flemming.
- Proved that broth culture of Penicillin nirayum has antibacterial property to gram positive bacteria by inhibit their cell wall synthesis.
- Most of penicillin are 6-aminopencillanic acid derivative.
- Streptomycin is an effective against mycobacterium tuberculosis and gram negative bacteria.

PENCILLIN

Penicillium chrysogenum spore suspension are inoculated onto wheat bran nutrient solution.

- Agitated for 1_2 days for to have a mycelial growth.
- Medium:lactose, caco3, glucose.
- PH:7.3 ,temp:73-81F
- 5-7 days for batch fermentation.

Three phases:

1: mycelial growth

2: consumption of lactose, mycelial mass increases, pH constant.secreting pencillin to the medium. 3:Antibiotic concentration decreases.

• Pencillin is a secondary metabolite so it produced during stationary phase.

*Using rotating vacuum filter, penicillin is separated from fungal cell.Fungal biomass is dried and used as a animal feed. Quality assurance and packaging



Biosynthesis of pencillin

- 1. Condensation of 3 aminoacid to form tripeptide
- 2. Biosynthesis of pencillin G
- 3. Transamidation
- These biosynthesis is encoded by gene penDE
 Pencillin is the first commercial product by aerobic submerged fermentation.

Streptomycin:

- used against TB
- Produced from Streptomycin griseus. (Actinomycetes)
- Inoculation to initiate the the fermentation in production tank.
- Medium:Soyabean meal, (N-source), glucose (C-source)
- Carried out at 28 °C,PH-7.3usally used :Hockenhul medium
- Process lasts for 5-7 days.

*Three phases;

1:Rapid growth of microbe, it's proteolytic activity produces NH3 to medium from soyabean meal causes increase in PH.

2:Little additional production of mycelium.streptomycin accumulate s.glucose and NH3 is consumed thus PH is constant.

3: Carbohydrates become depleted, production ceases.

 Recovery: streptomycin is absorbed onto charcoal and eluted with HCL Percipitated with acetone and purified by chromatography.

PRODUCTION OF STEROIDS

Why steroid productions are different from other production?

-CO₂H CO₂Me 8 steps HO **Chemical Synthesis** Deoxycholic acid (4) **Key intermediate** 37 steps **Overall vield: 1.6%** 29 steps Price: 200 \$/g (in 1946) **Combined Microbial**/ Cortisone (1) **Chemical Synthesis** 12 steps **Overall yield: 10.6%** 11 steps Price: 6 \$/g (in 1952) HO, Rhizopus arrhizus >85% Progesterone (2) 11α -OH-Progesterone (3)

OH

- Why chemical synthesis is so difficult?
 - Chemical conversion of deoxycholic acid to cortisone require 37 steps and extreme reaction condition,oxygen is needed to be introduced at 11 position
 - It is a difficult process
 - Microbes can introduce oxygen at this position in a single reaction.
 - Steroid transformation is actually occurring than production. Involoved in regulations of sexuality. Rhizopus Nigerians, Aspergillus species used in steroid production.
 - Microbial biotransformation include hydrogenation, dehydrogenation, oxidation and addition of side chain.

PRODUCTION

- Rhizopus Nigricans is grown in a fermentation tank
- Glucose or sucrose as carbon source and corn steep liquor as nitrogen source.
- Steroid to be transformed is first dissloved with desired solvent is Incorporated.
- In few hours or days steroid is transformed.
- Steroid is hydroxylase at number 11 to form 11-alpha hydroxyprogesterone.

RECOVERY

Extraction with methyl chloride, purified chromatography and recovered by crystallization.

PRODUCTION OF VITAMINS

- Commercially produced by using microorganisms like sterptomyces, acetobacter anaerobically or aerobically.
- VITAMIN B12
- Also called cyanocobalamine.
- Discovered by George whipple
- Major sources :egg,meat,poultry
- Soluble in water, ethanol
- Red coloured vitamin
- One of 8 B vitamin
- Production requires 3-5 days

INDUSTRIAL PRODUCTION BY FERMENTATION

INNOCULUM: pure **Streptomyces olivaceus** is inoculated and grown in 100-250ml of inoculum medium.

MEDIUM: carbohydrate, protien, cobalt.sterillization of medium by batch or continous culture

- Temperature:80 F
- PH:5 with H₂SO₄ and reducing agent Na₂SO₄
- Optimum aeration, excess aeration may causes foaming
- Yield: 1-2 mg per litre in fermented broth

<u>RECOVERY:</u> Cobalamine associated mycelium boiling mixture liberate cobalamine quantitatively from mycelium filteration is done to remove mycelium ,filtrerated broth then treated with cyanide.

- Adsorption chromatography is done by activated charcoal.
- Elution:process of extracting material from another by washing with solvent like water. Evaporation upto dryness.

<u>USES</u>

- Regulates the overproduction of igE
- Reduces depression
- Stimulate the normal functioning of nervous system.
- animal protein can be replaced by less expensive vegetable proteins.
- If the vegetable protein is fortified with vitB12 has a role in biological nitrogen fixation.

DEFICIENCY

- Causes muscle pain
- Memory loss
- Causes infertility

RIBOFLAVIN, VITAMIN B2

- Kuhn, Guriga and eager isolated riboflavin from the whey of milk.
- Ashbya gossypi is used as microbes.
- It has 400 time more production rate.
- Clostridium sps, Candida sps belong to ascomycetes can be employed also.
- Fermentation can be done in a single step so that can save cost.

Medium : corn steep, molasses,

collagen, siya oil .

Fermentation: submerged aerobic,3 days at 20-28°C

- PH:6-7.5
- 3-6 g/litre production


RECOVERY

- Done by two process
- 1. Evaporation of whole broth
- 2. Drying by drum drier.

<u>USES</u>

- Help in the breakdown of carbohydrate , protein and there by provide energy
- Good for overall health
- Promote growth

DEFICIENCY

- Causes nutritional disorders
- Crack in skin corner of mouth
- Fissure of lips

PRODUCTION OF AMINO ACID

3 major aminoacid produced industrially

- L-Lysine
- L-glutamic acid
- Submerged aerobic fermenter
- Batch or fed batch fermentation
 process
- Microrganism: Cornybacterium glytamicum
- Medium: carbon source :cane molasses,nitrogen source:soyabeen ,mineral salts,antifoaming agents :PEG
 - Optimum PH:7.2,temperature:35-37 c
- Recovery :by ion exchange method ,lysine is adsorbed and eluted by washing it with ammonia solution.



- Corynebacterium glutamicum produces Llysine.
- Medium contain glucose, ammonium acetate.
- Recovery:after 30-35
 hours.
- All most all process steps are same for all.
- Application of L-Lysine
- Flayour enhancer
- Poultry feed
- As nutritional supplement



PRODUCTION OF ORGANIC ACID

Production of Lactic acid:

- First discovered by Scheele(1789) from sour milk.
- Fermentation: racemic mixture is formed due to enzyme racemase. Due to it it becomes optically inactive.
- Homofermentative process: Involves the bacterium Lactobacillus delpruckii.
- Utilizes the Emp pathway to produce pyruvic acid which then reduced to lactic acid by lactate dehydrogenase.

- Heterofermentative:involves production of ethanol ,lactic acid ,CO₂ by Leuconostoc mesensteroids.
 - Medium,:sugar molasses,lactic acid is corrosive and thus metals are avoided.
- Recover:by CaCO₃ and H₂SO₄ , it formed as crystal.

<u>USES</u>

- Beverages
- Food
- Pharmaceutical
 - Agriculture
 - Metal Industry

PRODUCTION OF CITRIC ACID

- First produced by John and Edmund.
- Fermentation: Aspergillus niger is used
- Koji process-Japanese process sweet potato starch as solid substrate.
- Submerged fermentation:fungal mycelium grows through out.
- Medium: carbon source:molasses,PH:2,temperature :30c
- Batch or fed batch is used.



Used

- Sold as anhydrous crystalline.
- Used in soft drinks, jams , jelly, wine, candies, frozen fruit.
- Used in blood transfusion and as a effervescent product.
- In cosmetics.

PRODUCTION OF ALCOHOL

- Known as the nature of process.
- Louis Pasteur showed yeast is fundamental to process.
- Saccharomyces cerivisea, candida brassicae been employed.
- 97% conversion to ethanol by both sucrose and glucose.

Limitations of yeast

Whole biomass can't be converted to ethanol.
There exit a limitation.

 Zymomonas has merit over yeast in having the osmotic tolerance to higher sugar concentration.



Preparation of medium

1.Starch contained substrate.

- 2.Juice from sugar cane or molasses or sugar beet.
- 3.Waste product from wood or processed wood.
- Yeast doesn't contain amylases.

Fermentation

- Is a action of yeast in sugar solution and breakdown to alcohol and CO₂.
- CO₂ escapes and fermented beverages exist .Eg:wine,beer

Formula = (C6 H12 06)N + yeast → 2C2H5OH + 2CO2

Recovery

- By distillation 95% is obtained.
- Require an azeotropic mixture of ethanol,water, benzene,so after distillation absolute alcohol is obtained.
- Water evaporate at 100°C and ethyl alcohol at78°C.
- This is used to separate ethyl alcohol from water and residue.
- It is of Dutch origin.



PRODUCTION OF BEER

- For more than 6000 years.
- Brewing origin from Mesopotamia.mainly from grains about 40%.



 Grains are easy for transportation, easy at separation of wort.

- Beer is usually a batch fermentation.
- Product of fermentation of barley grain by yeast.
- Process is known as brewing.



1. MALTING

- Barley seeds are allowed to germinate.
- Naturally occurring amylases convert the grain starch to sugars of maltose.

Process called malting and digested grain as malt.



2:MASHING

- Mash the grain with water and remove the fluid portion called wort.
- Dried petals of Humulus lupulus called hops are added to flavour, colour, also to prevent contamination of wort due to two antimicrobial substance in petals.
- Now add yeast in large amount after filtering.









of different malt types in beer recipes.

Percent of Recipe

About yeast

- Common Saccharomyces cerevisiae.
- Impart uniform cloudiness.
- Top yeast: cloudiness is carried to top of fermentation vat by forming CO₂ and product is ale.
- 2. Bottom yeast: ferment the beer more slowly and produce light beer with alcohol less than ale and product is lager beer.

Eg: Saccharomyces carlsbergensis,uk.



- After one week, young beer, transferred to vat for 1°, 2° ageing about 6 months.
- Keg beer:left in beer.
- Canning, pasteurize at 140°F for 13 min.
- Rest used as animal feed , pressed to form scp.
 - 4% alcoholic content.



Commonly rice wine

From steamed rice.

Convert to sugar by Aspergillus oryzae

Ferment by saccharomyces up to 14% alcohol.



THE ART OF **SAKE MAKING**

HARVESTING

Rice for sake is different from the rice used for eating.



STEAMING

Steaming gives the rice a harder outer shell and a softer core.



SOAKING

The soaking time varies from a few minutes to several hours, depending on the type of rice and the taste components sought for the sake.

POLISHING

The longer the polishing,

the cleaner the sake flavour.

WASHING

Also known as the second polishing process.

KOJI MAKING

Cultivation of koji mold. Koji converts the rice starch into sugar (glucose).



Steamed rice







PRODUCTION OF WINE

- Fermentation of fruit juice by yeast.
- Commonly two kind of grapes, red wine from red grapes, white wine from white grapes.
- Common saccharomyces ellipsoideus, a variety of saccharomyces cerivisea.

Wine Production : Main Steps



- 1. Viticulture
- 2. Harvesting
- 3. Stemming/Crushing
- 4. Fermentation
- 5. Draining
- 6. Pressing
- 7. Mixing
- 8. Clarification
- 9. Aging
- 10. Bottleing



- Stemming is the removal of stem of grapes
- Crushing is the pressing to squeeze the fresh juice
- Crushed grape called must.
- So2 is used to control the process.
- Sodium or potassium meta bisulphate is added to check the undesirable microorganism.





- <u>Stemming</u> is the removal of stem of grapes
- <u>Crushing</u> is the pressing to squeeze the fresh juice. Crushed grape called must.
 SO₂ is used to control the process. Sodium or potassium meta bisulphate is added to check the undesirable microorganism.
- <u>Draining</u>: liquid wine is drained from vat without being pressed and go into barrel.called free run wine.
- <u>Pressing</u>: remaining pulp ,after draining, is pressed to squeeze out.
- <u>Mixing</u>:free run wine and press wine from same source is mixed to obtain desirable balance.
- <u>Clarification</u>: stabilising of fermentation, remaining solid are removed.



- For red wine, fermentation's carried at 24-27°C for 3-5 days.
- White wine takes 1-2 weeks at 10-21°C.
- MICROBIAL DETERIORATION
 - Acetification, glucose may oxidise may given sweet sour taste to wine.done by Acetobacter aceti.
- The fermentation to glycerol called amerture.

TYPES OF WINE

- Still wine : wine in which no CO₂ is produced during fermentation.
- Carbonated wine : wine in which considerable amount of CO₂ is produced.
- Sherry: popular in France, made from ripe and dried grapes with high sugar content, grapes infected with Botrytis cinerea are selected for sherry production.
- Dry wine : most or all sugars is metabolised.
- Sweet wine: fermentation is stopped before entire sugar is consumed.
- Fortified wine : brandy,rum are added to get 22% alcohol.

DISTILLED SPIRITS

- Considerably more alcohol than beer and wine.
- Shown by proof number which is twice the actual percentage of alcohol.
- Process is same but after fermentation alcohol is collected by distillation.
- During maturation, chemicals like aldehyde, ethers and volatile acid are added.
- Then alcoholic content is standardized by diluting it with water.

DISTILLED LIQUID TYPES

- 1. <u>RUM:</u> obtained by distillation from alcoholic fermented sugar cane,syrup,molasses.
- 2. <u>WHISKEY:</u>obtained_after distillation from saccharified and fermented grain mashes.prepared by using wheat,corn,rye.
- 3. <u>BRANDY</u>: obtained from grape wine after distillation.also from apple, peach, apricot.
- 4. <u>NEUTRAL SPIRIT :</u> as vodka made from potato starch and left unflavoured and gin flavoured with juniper oil.
- 5. <u>Tequila :</u> obtained by distillation from alcoholic fermented agave

The Basic Process of Whiskey Making



Preparation

Various grains are ground and cooked. Barley is malted, a process of soaking the barley and spreading it for about three weeks, allowing it to sprout, and drying and heating it.



Mashing

The cooked grain and malted harley are added to warm water, which converts into a liquid known as mash.



Fermenting

The mash is added to a fermentation tank, along with yeast. The yeast converts the sugar to alcohol. After three or four days, the resulting liquid is about 10% alcoholic and is known as distiller's beer, or wash.



Distilling

The wash is heated to the point where the alcohol turns to vapor, but the water remains liquid. The alcohol is then collected in a second container. This process is repeated to produce "high wine" or "new whiskey."



Aging

Water is added to the high wine, which is is aged in wooden barrels, usually made from charred white oak. Here the whiskey ages at least three or four years, and some are aged up to ten or fifteen years.



Bottling

The resulting whiskey is stored in glass bottles, which do not react with the whiskey's flavor.



Rum

Gin




Vermouth

Brandy



Whiskey





PRODUCTION OF VINEGAR

- French word vinaigre means sour wine.
- Major two events, conversion of sugar to ethanol by yeast (saccharomyces cervisiae, ethanol is oxidized to acetic acid by acetobacter and glucanobacter called acetification.



<u>SUBSTRATE</u>: It Can Be A Fruit Juice, starchy vegetables, malted cereals such as wheat, barley, corn, or it can be sugar cane, honey.





METHODS OF PREPARATION

1.<u>SLOW METHOD</u>

- Oldest method, still used in some European countries.
- An initial natural alcoholic fermentation achieve 11-13% alcohol.
- Acetic bacteria are seeded to convert acetic acid.
- Introduced by France.



Slow process

2:QUICK PROCESS (ORLEANS PROCESS)

- A barrel is filled about 1/4 th full with raw vinegar from previous run to supplement active vinegar bacteria.
- Then substrate is added.
- Takes several months to complete at 21-29°C.
 - Improved quality of vinegar is produced. Introduced by German.

3: NATURAL FERMENTATION

- Fermentation take place naturally.
- Sugar or coconut sap is placed in earthen jars.



 Vinegar Generator: used to increase the rate of acid production.

- Alcohol liquid is trickled over the surface of film of acetic acid bacteria.
- The film of bacteria is on the wood chips.
- In modern ,production of vinegar through sub merged culture reactor.
- The final product is kept to a age for final taste.







- To preserve food.
- As deodorant
- To make salad
- As a facial toner.
- To trap fruit flies
- To boil better eggs
- To wash fruits and vegetables
- To whiten teeth.

