BHARATHIDASAN UNIVERSITY
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Programme : M.Sc., Biotechnology (Marine)
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UNIT IV
MICROBIAL FERMENTATION AND PRODUCTS

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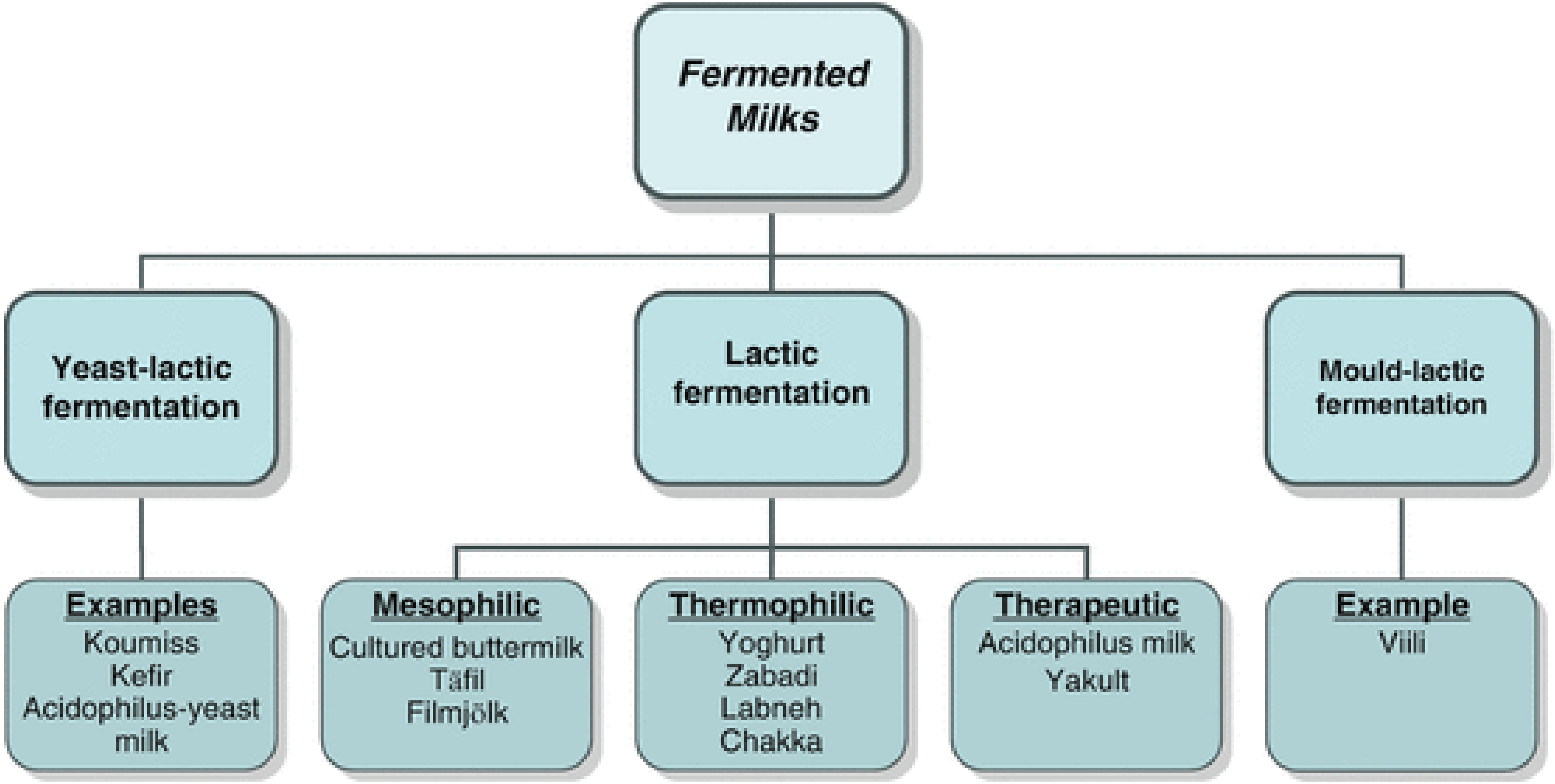
Microbial Fermentation

Fermentation is a metabolic process in which an organism converts a carbohydrate, such as starch or a sugar, into an alcohol or an acid.

These are three distinct types of fermentation that people use.

- Lactic acid fermentation. Yeast strains and bacteria convert starches or sugars into lactic acid, requiring no heat in preparation. ...
- Ethanol fermentation/alcohol fermentation. ...
- Acetic acid fermentation.

Fermented Milk Products



Fermented Milks

Yeast-lactic fermentation

Lactic fermentation

Mould-lactic fermentation

Examples
Koumiss
Kefir
Acidophilus-yeast milk

Mesophilic
Cultured buttermilk
Täfil
Filmjölk

Thermophilic
Yoghurt
Zabadi
Labneh
Chakka

Therapeutic
Acidophilus milk
Yakult

Example
Viili

1. Buttermilk

- There are two types of buttermilk.

- Traditional buttermilk is a thin, cloudy, slightly tart but buttery-tasting liquid that's left after cream is churned to make butter. These days, however, it is more commonly sold as a thick liquid produced commercially by adding an acidifying bacteria – and sometimes flavoring and thickening agents – to milk.

- This commercial product can be thought of as a gentler, thinner yogurt, with any buttery flavour likely added.

Preparation

- Buttermilk is made by heating skim or low-fat milk to 88 °C (180 to 190 °F) for 30 minutes or 90 °C (195 °F) for two to three minutes.
- This heating process is done to destroy all naturally occurring bacteria and to denature the protein in order to minimize wheying off (separation of liquid from solids).
- The milk is then cooled to 22 °C (72 °F), and starter cultures of desirable bacteria, such as ***Streptococcus lactis*, *S. cremoris*, *Leuconostoc citrovorum*, and *L. dextranicum***, are added.
- These organisms convert lactose into lactic acid, a process that develops buttermilk's acidity and unique sour flavour; the different types of bacteria may be used singly or in combination to obtain the desired flavour.
- The addition of these bacteria makes buttermilk easier to digest by lactose-intolerant consumers.
- The ripening process takes about 12 to 14 hours (overnight). At the correct stage of acid and flavour, the product is gently stirred to break the curd, and it is cooled to 7.2 °C (45 °F) in order to halt fermentation. It is then packaged and refrigerated.

2. Yogurt

- Yogurt is known and consumed in almost all parts of the world. Various flavours and sweetening may be added, or natural yogurt may be mixed with fresh fruits or vegetables. A salad of yogurt, cucumbers, and spices is served in India (*raita*) and several Middle Eastern countries (*jajik*). Yogurt is also used in soups and sauces.
- Commercial dairies usually add milk solids to cow's milk to make yogurt with a custardlike consistency.
- Concentrated sterilized milk is inoculated with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*; sometimes *L. acidophilus* or a lactose-fermenting yeast is also added.
- This inoculated milk is then incubated four or five hours at about 43–44 °C (110–112 °F) until curd forms.

Preparation

- Yogurt is made in a similar fashion to buttermilk and sour cream, but it requires different bacteria and temperatures. Whole, low-fat, or skim milk is fortified with nonfat dry milk or fresh condensed skim milk, in order to raise the total solids to 14 to 16 percent.
- The mixture is heat-treated as for buttermilk and then cooled to 45.6 to 46.7 °C (114 to 116 °F). At this point a culture of equal parts *Lactobacillus bulgaricus* and ***Streptococcus thermophilus*** is added to the warm milk, followed by one of two processing methods.
- For set, or sundae-style, yogurt (fruit on the bottom), the cultured mixture is poured into cups containing the fruit, held in a warm room until the milk coagulates (usually about four hours), and then moved to a refrigerated room.
- For blended (Swiss- or French-style) yogurt, the milk is allowed to incubate in large heated tanks. After coagulation occurs, the mixture is cooled, fruit or other flavours are added, and the product is placed in containers and immediately made ready for sale.

3. Kefir

- Kefir (pronounced kuh-feer) is a fermented milk drink, made from a combination of bacteria and yeast fermentations, that originally hails from the Caucasus area (the mountainous region dividing Asia and Europe). It is usually made from cow's milk, but can also be made from goat or sheep's milk, as well as non-dairy milk.
- Traditionally, kefir is manufactured using cow, ewe, goat, or buffalo milk.
- In kefir grains the main polysaccharide is kefiran, which is a heteropolysaccharide composed by equal proportions of glucose and galactose and is mainly produced by ***Lactobacillus kefiranofaciens.***
- Kefir grains have a complex composition of microbial species such as the predominance of lactic acid bacteria, acetic bacteria, yeasts, and fungi.

- The microbial composition may vary according to kefir origin, the substrate used in the fermentation process and the culture maintenance methods. Tibetan kefir, which is used in China, is composed of *Lactobacillus*, *Lactococcus*, and yeast.
- Additionally, acetic acid bacteria have been identified in Tibetan kefir, depending on the region in China from where it was obtained (Gao et al., 2012), additionally, Tibetan kefir composition differs from that of Russian kefir, Irish kefir, Taiwan kefir, Turkey fermented beverage with kefir; however, it is known that this microbial diversity is responsible for the physicochemical features and biological activities of each kefir

Types

Grains

- Kefir is made using "starter" grains (a complex combination of bacteria, yeasts, milk proteins, and complex sugars), which help to ferment the milk. These kefir grains are not like rice grains or other regular kinds of grains—they do not have any gluten in them.

Milk

- Kefir milk tastes a bit more tart, tangy, and fresh than yogurt, and you can find it in endless delicious fruity flavor options. However, unlike yogurt, it is a liquid and you drink it rather than eat it with a spoon.

Water

- Water kefir is a probiotic drink that's made with water kefir grains. Water kefir grains are used to culture sugar water, juice, or coconut water. The amount of sugar in water kefir depends on how long it is allowed to culture (as it cultures, the grains take the sugars and convert them into carbon dioxide, yeasts, bacteria, and acids).

Cultures

- In addition, *Bifidobacterium* sp., *Lactobacillus* sp. and probiotic yeast (*Saccharomyces boulardii*) may be used as adjunct cultures when blended with kefir grains.
- In that way, *Lactobacillus paracasei* ssp. *paracasei*, *Lactobacillus acidophilus, Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus plantarum*, and *L. kefiranofaciens* are predominant species.

Production

- There are three main ways of producing kefir (I) the artisanal process, (II) the commercial process by the Russian method and (III) the commercial process using pure cultures.
- The traditional artisanal production involves milk inoculation with a variable amount of grains and fermentation for a period between 18-24 h at 20-25 ºC.
- At the end of the fermentation process the grains are sieved and can be used for a new fermentation or kept (1-7 days) in fresh milk, while the kefir beverage is stored at 4 ºC, ready for consumption.
- The initial inoculum concentration of the grains (grain/milk proportion) affects the pH, viscosity, final lactose concentration and the microbiological profile of the final product.
- Agitation during fermentation also influences kefir microbial composition, favoring the development of homofermentative lactococci and yeast. Incubation at temperatures above 30 ºC stimulates the growth of thermophilic LAB, while being a disadvantage for yeast growth and mesophilic LAB.

- The second method, known as the "Russian method", allows for the production of kefir on a larger scale, and uses a process of fermentation in series, from the percolate resulting from the first fermentation of the grains (fermented without the grains or mother culture).
- Different methods can be used in the industrial process of kefir production, but all based on the same principle.
- The milk is inoculated with pure cultures isolated from kefir grains and commercial cultures.
- The maturation phase can be performed or not, consisting of maintaining the kefir at 8-10 ºC for up to 24 h, to allow microorganism, primarily yeast, growth, contributing to the specific flavour of the product.
- Omission of this step is associated with development of atypical flavour in kefir.
- During storage, the CO2 production by yeast or heterofermentative LAB can cause bloating in the product package, a fact that should be considered in the choice of packaging.

4. Tofu

- **tofu**, also called **bean curd**, soft, relatively flavourless food product made from soybeans. Tofu is an important source of protein in the cuisines of China, Japan, Korea, and Southeast Asia. It is believed to date from the Han dynasty (206 BCE–220 CE).

Preparation

- Tofu is made from dried soybeans that are soaked in water, crushed, and boiled. The mixture is separated into solid pulp (*okara*) and soy "milk."
- Salt coagulants, such as calcium and magnesium chlorides and sulfates, are added to the soy milk to separate the curds from the whey. In some cases, acid coagulants, such as citric acid or glucono delta-lactone, may be used.
- The soy milk is poured into molds to allow the carbohydrate-laden whey to drain off. The resultant soft cakes are cut into squares and stored under water until sold, in bulk or in individual water-filled tubs. Tofu can be made extra soft, soft (silken), firm, or extra firm, depending on the method of production. Dried tofu, which does not require refrigeration, is also sold.

- Lactic acid bacteria species predominated in correctly preserved fresh tofu and bacterial species able to grow after thermal abuse mostly derived from the raw materials and belonged to heat labile species, thus suggesting that a more efficient heat treatment can increase the shelf-life of the product.

Fermented vegetables

- Sauerkraut
- Kimchi
- Olives
- Gundruk
- Khalpi
- Sinki

Prebiotics

- A non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health”.

- They can feed the intestinal microbiota, and their degradation products are short-chain fatty acids that are released into blood circulation, consequently, affecting not only the gastrointestinal tracts but also other distant organs.

- Fructo-oligosaccharides, trans-galacto-oligosaccharides and galacto-oligosaccharides are the important groups of prebiotics with beneficial effects on human health.

Fermentation of prebiotics by gut microbiota produces short-chain fatty acids (SCFAs), including lactic acid, butyric acid, and propionic acid. These products can have multiple effects on the body.

- **Fructans**

This category consists of inulin and fructo-oligosaccharide or oligofructose. Their structure is a linear chain of fructose with β(2→1) linkage. They usually have terminal glucose units with β(2→1) linkage. Inulin has DP of up to 60, while the DP of FOS is less than 10.

- **Galacto-Oligosaccharides**

Galacto-oligosaccharides (GOS), the product of lactose extension, are classified into two subgroups:

(i) the GOS with excess galactose at C_3, C_4 or C_6

(ii) the GOS manufactured from lactose through enzymatic trans-glycosylation. The end product of this reaction is mainly a mixture of tri- to pentasaccharides with galactose in β(1→6), β(1→3), and β(1→4) linkages. This type of GOS is also termed as trans-galacto-oligosaccharides or TOS.

GOSs can greatly stimulate *Bifidobacteria* and *Lactobacilli*. *Bifidobacteria* in infants have shown high incorporation with GOS. *Enterobacteria*, *Bacteroidetes*, and *Firmicutes* are also stimulated by GOS, but to a lesser extent than *Bifidobacteria*

- Starch and Glucose-Derived Oligosaccharides

There is a kind of starch that is resistant to the upper gut digestion known as resistant starch (RS).

RS can promote health by producing a high level of butyrate; so it has been suggested to be classified as a prebiotic .

Various groups of *Firmicutes* show the highest incorporation with a high amount of RS.

An in vitro study demonstrated that RS could also be degraded by *Ruminococcus bromii*, and *Bifidobacterium adolescentis*, and also to a lesser extent by *Eubacterium rectale* and *Bacteroides thetaiotaomicron*. However, in the mixed bacterial and fecal incubations, RS degradation is impossible in the absence of *R. bromii.*

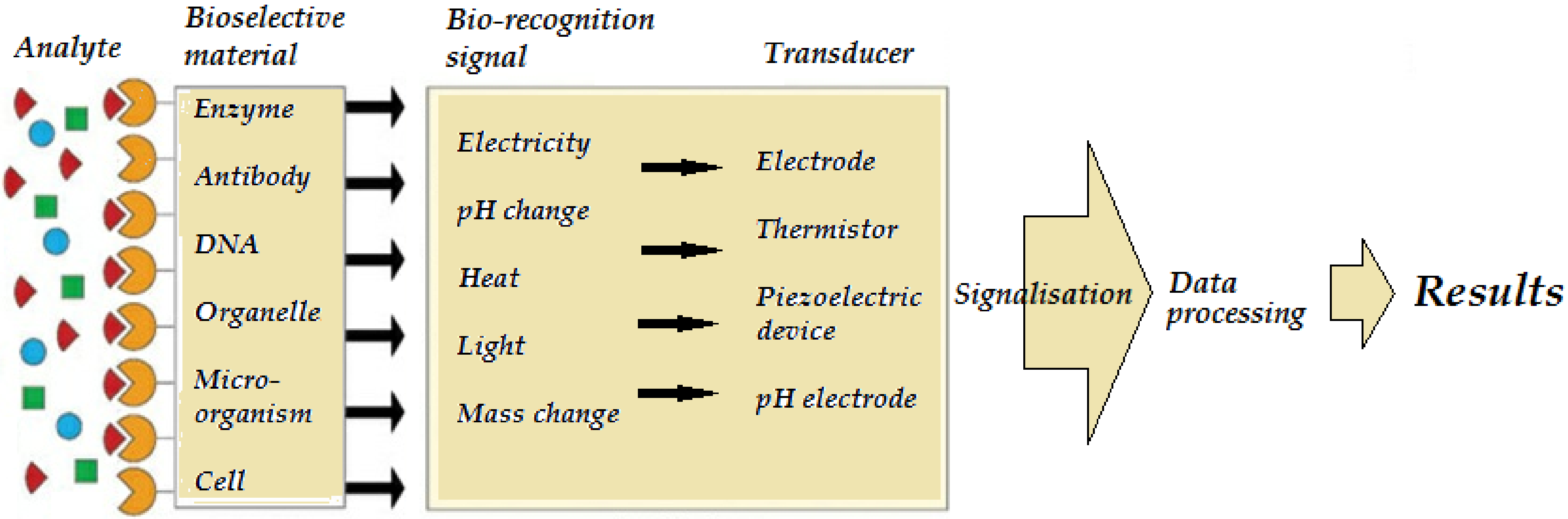
Biosensor

A Biosensor is an analytical device that detects changes in Biological processes and converts them into an electrical signal. The term Biological process can be any biological element or material like enzymes, tissues, microorganisms, cells, acids, etc.

these sensors are advanced in the conditions of selectivity as well as sensitivity.

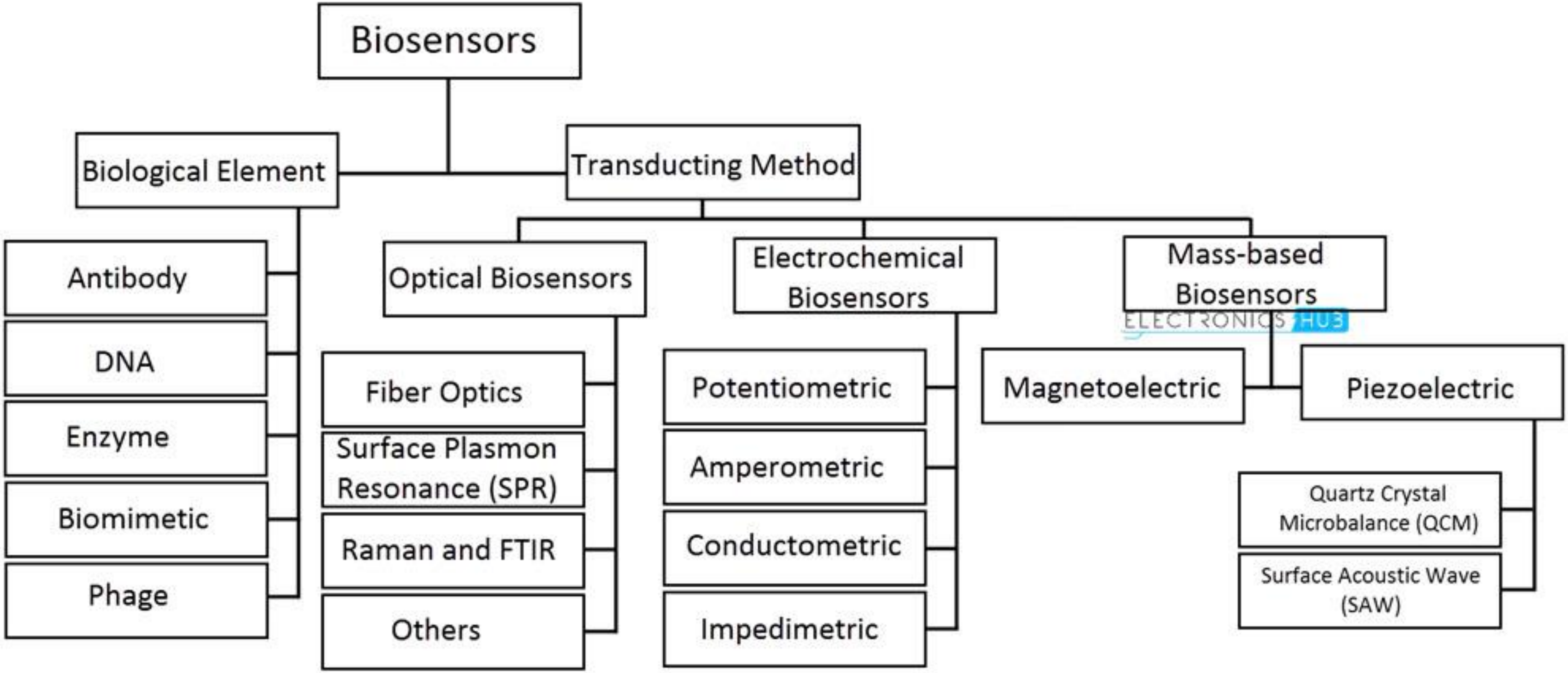
there will be an electronic circuit which consists of a Signal Conditioning Unit, a Processor or Microcontroller and a Display Unit.

Working principle and components



Types

- the biosensors based on method of **transduction** are again divided into three types. They are:
- Mass based Biosensors
- Optical based Biosensors
- Electrochemical Biosensors



- Biosensors
  - Biological Element
    - Antibody
    - DNA
    - Enzyme
    - Biomimetic
    - Phage
  - Transducting Method
    - Optical Biosensors
      - Fiber Optics
      - Surface Plasmon Resonance (SPR)
      - Raman and FTIR
      - Others
    - Electrochemical Biosensors
      - Potentiometric
      - Amperometric
      - Conductometric
      - Impedimetric
    - Mass-based Biosensors
      - Magnetoelectric
      - Piezoelectric
        - Quartz Crystal Microbalance (QCM)
        - Surface Acoustic Wave (SAW)

ELECTRONICS HUB

Components

i. Sensor or **detector:** The first segment is the sensor or detector which is a biological component. it is a biochemical receptor. It interacts with the analyte and signal the change in its composition as electrical signal.

ii. Transducer: The second segment is the transducer and it is a physical component which amplifies the biochemical signal received from detector, alters the resulting signal into electrical and displays in an attainable way.

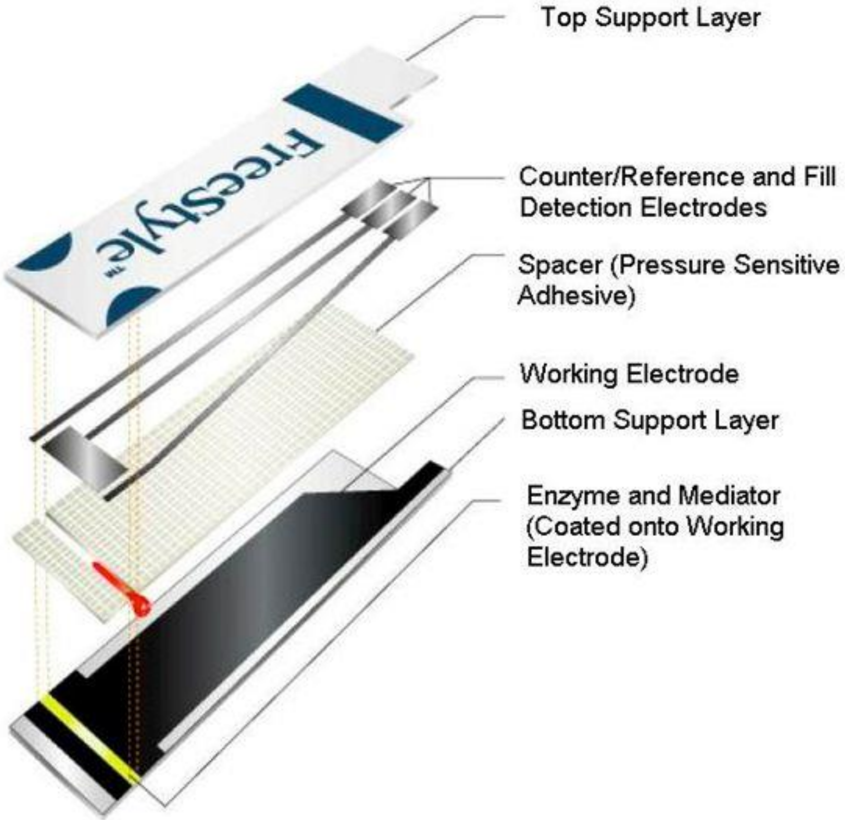
iii. Electrical circuit: It is the associated part which consists of Signal Conditioning Unit, a Processor or Micro-controller and a Display Unit.

Examples

- 1. The pregnant test kits are antibody-based sensors for the detection of HCG, a biomarker for pregnancy.
- 2. The first generation of glucose biosensors used atmospheric oxygen as a substrate and detected the production of hydrogen peroxide. The second-generation biosensors replaced oxygen with other electron mediators whose function was to carry the electrons from the enzyme to the electrode. The third-generation biosensors were totally reagent-less. The electron mediators were no longer used and the enzyme directly transferred the electrons to the electrodes.

Test Strips

- When blood added, glucose is oxidized by enzyme coated on working electrode
- Voltage applied between working and reference electrode
- Measure current between working and reference electrode



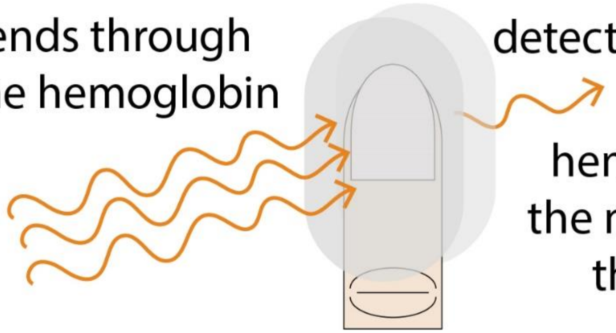
3.

Pulse oximetry

*oxy*hemoglobin and *deoxy*hemoglobin prefer different colors of light, & you can use this to measure **oxygen saturation** if you play your cards right!

send in light

light source sends through light one of the hemoglobin forms likes



see how much makes it through

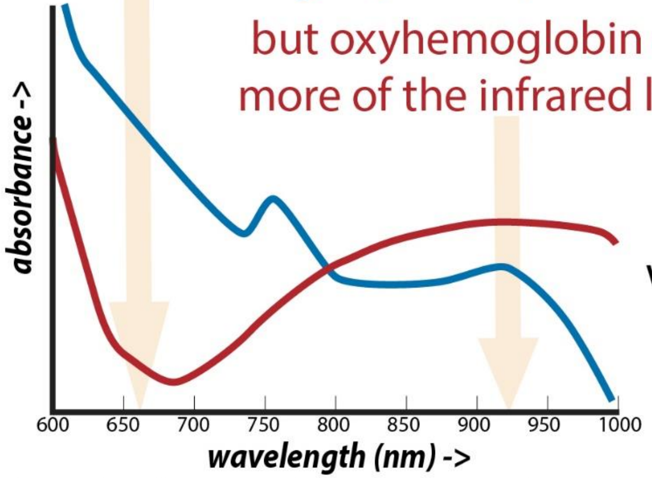
detector measures transmission

the more of that hemoglobin form there is, the more light is absorbed & the less is transmitted

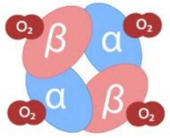
pulse oximeters measure transmission at 2 wavelengths and then looks at the ratio to calculate oxygen saturation

deoxyhemoglobin absorbs more of the reddish light (660nm)

oxyhemoglobin does not absorb that red light (so blood looks red!)



but oxyhemoglobin does absorb more of the infrared light (940nm)



so you measure both wavelengths & and compare to figure out the proportion of hemoglobin bound to oxygen (the oxygen saturation)

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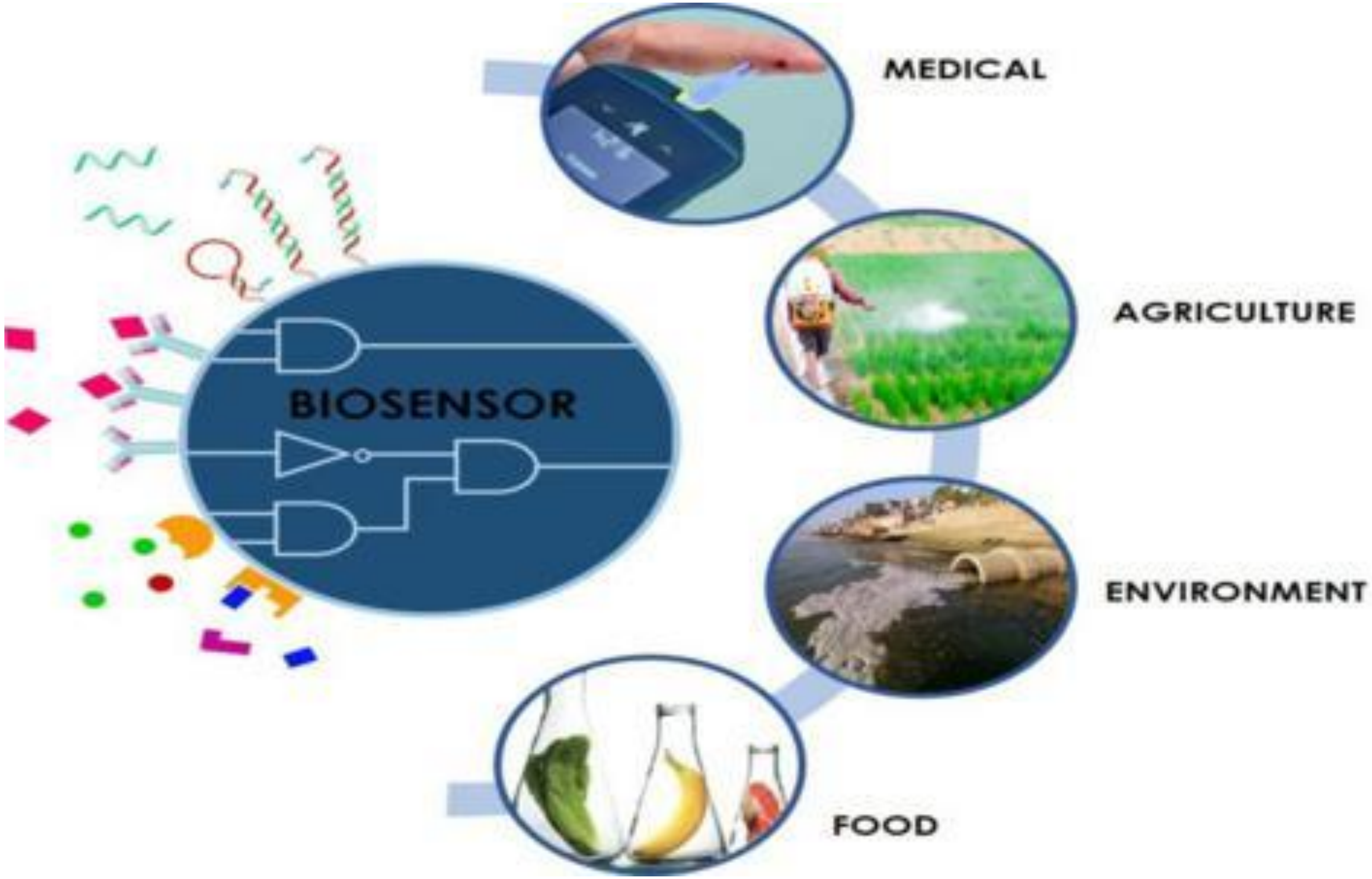
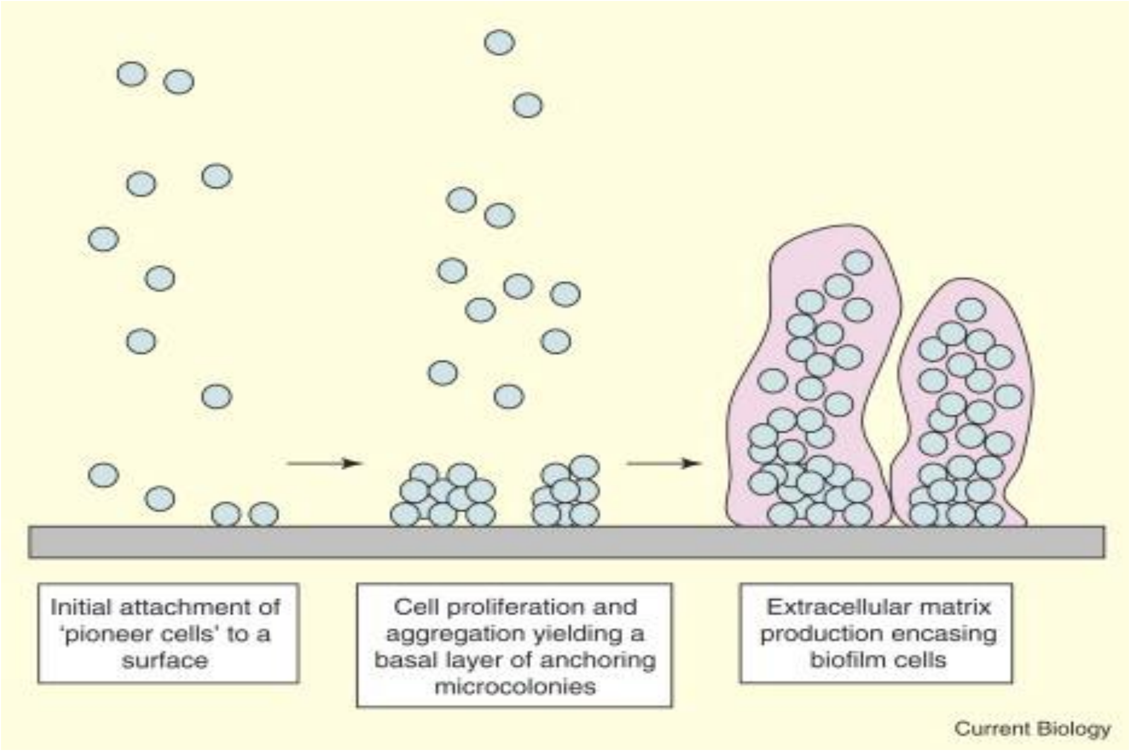
Applications

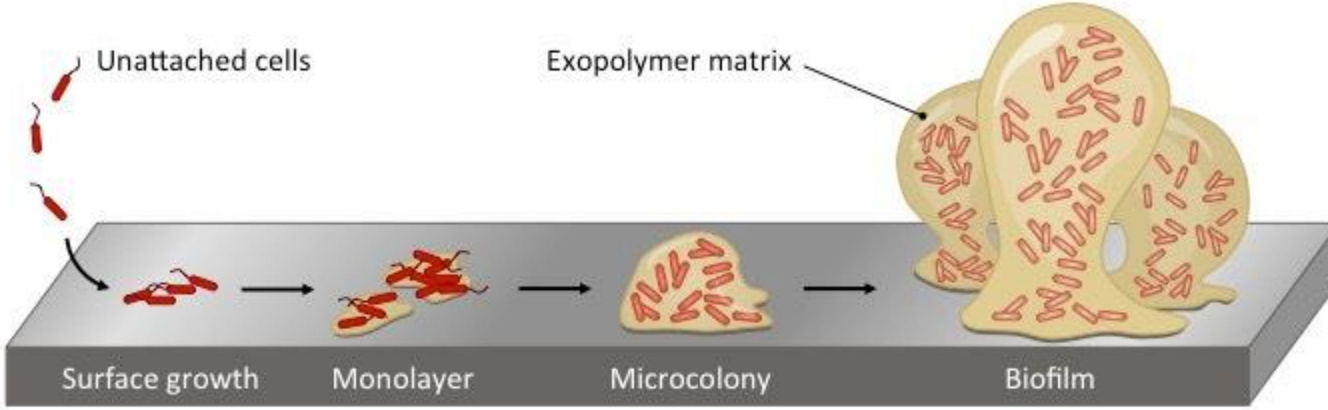


Biofilm

- Biofilms are a collective of one or more types of microorganisms that can grow on many different surfaces. Microorganisms that form biofilms include bacteria, fungi and protists.
- Biofilm formation begins when free-floating microorganisms such as bacteria come in contact with an appropriate surface and begin to put down roots, so to speak. This first step of attachment occurs when the microorganisms produce a gooey substance known as an extracellular polymeric substance (EPS),
- An EPS is a network of sugars, proteins and nucleic acids (such as DNA). It enables the microorganisms in a biofilm to stick together.

(1)bacterial attachment to a surface,
(2) microcolony formation,
(3) biofilm maturation and
(4) detachment (also termed dispersal) of bacteria which may then colonize new areas





- Initial Attachment

Initial attachment occurs when a material surface is exposed to an aqueous medium (e.g., water, blood) and is coated by polymers from that medium. This is known as the conditioning layer; it is organic, forms within minutes of exposure, and continues to grow for several hours. The cells that comprise the conditioning film attach quickly and efficiently to hydrophobic, nonpolar surfaces, such as plastics. In addition, initial attachment and colonization increase as surface roughness increases. This is due to the larger surface area on rougher surfaces (Donlan, 2002). Planktonic microorganisms instinctually attach themselves to this conditioning layer to become part of the developing biofilm (Cogen et al., 2004

Irreversible Attachment

The second stage, irreversible attachment, begins once the microorganisms start producing the extracellular polymeric substance, EPS, which represents the “house of the biofilm cells” (Flemming et al., 2007). The polyhydroxyl groups in EPS anchor the bacteria in the biofilm to the surface through hydrogen bonding (Kjelleberg et al., 2007). At this time, the microbes can no longer move away from the surface. It has been determined that mature biofilms are anchored to their place until the final stage of growth (Kolari et al., 2001).

The Cell Matrix

The EPS is comprised of a wide variety of proteins, glycoproteins, glycolipids, and extracellular DNA. The biopolymers in the extracellular polymeric substance are highly hydrated and form a matrix that holds the biofilm together and retains water. This matrix interacts with the environment to provide nutrients for biofilm organisms. Micro-colonies are formed within the EPS during the maturation stages of development. The colonies are separated by pores and water channels, which function as pathways for nutrients and oxygen to flow through, and nourish the biofilm (Flemming et al., 2007

Maturation

During the maturation stages, the biofilm’s main goal is to grow in three dimensions. This is achieved by picking up debris in the surrounding environment, such as sand, and by recruiting new planktonic bacteria. The biofilm also grows in these stages through reproduction, which occurs regularly in the micro-colonies.

Dispersal

The final stage of development is dispersal, or shedding of cells. This stage is sometimes referred to as “expansion”. While many bacterial cells disperse from biofilms by passive processes, bacterial biofilms can also experience active dispersal events in which sessile, matrixencased biofilm cells convert to free-swimming planktonic bacteria (Webb, 2007). Quorum sensing, commonly referred to as cell-to-cell signaling, is essential to biofilm dispersal (Donlan, 2002). Through quorum sensing, bacterial populations will activate dispersion only when they are able to sense that their population is numerous enough to make it advantageous. For example, one study observed a cellular division of labor through quorum sensing, where one group of cells stayed attached to the surface and made nutrients available to the second group, which reproduced and released daughter cells to the surrounding water (MSU Center for Biofilm Engineering, 2008).

Why to form?

- The slimy EPS covering can act as a protective barrier.
- It can help prevent dehydration or act as a shield against ultraviolet (UV) light.
- Also, harmful substances such as antimicrobials, bleach or metals are either bound or neutralized when they come into contact with the EPS.

Application

1. **Bioremediation**

Sometimes, biofilms are useful. "Bioremediation, in general, is the use of living organisms, or their products — for example, enzymes — to treat or degrade harmful compounds. He noted that biofilms are used in treating wastewater, heavy metal contaminants such as chromate, explosives such as TNT and radioactive substances such as uranium. "Microbes can either degrade them, or change their mobility or their toxic state and therefore make them less harmful to the environment and to humans,

2. Nitrification using biofilms is one form of wastewater treatment. During nitrification, ammonia is converted to nitrites and nitrates through oxidation. This can be done by autotrophic bacteria.

3. Microbial fuel cells use bacteria to convert organic waste into electricity. The microbes live on the surface of an electrode and transfer electrons onto it, ultimately creating a current. Also that bacteria powering microbial fuel cells break down food and bodily wastes. This provides a low-cost source of power and clean sustainable energy.

4. Microorganisms in biofilm matrix could positively affect quality characteristics of food products such as texture, biochemical composition and sensorial properties *via* the production of specific secondary metabolites.

5. Waste water treatment

Secondary Treatment Secondary treatment of wastewater employs biological processes and the use of microorganisms to rid the water of any organic compounds that may still be present. This stage simulates what actually happens in nature, when microorganisms break down organic wastes. There are three main approaches that can be used in this stage:

fixed film, suspended film and lagoon systems (Mancl, 2009).

- Fixed Film In a fixed film system, microorganisms grow on rocks, sand, or plastic, to create a film. They grow on these surfaces by feeding off the organic matter and nutrients in the wastewater that flows over them. There are three main fixed film systems that are commonly used today: trickling filters, rotating biological contactors, and sand filters (Mancl, 2009).
- Suspended Film Suspended film systems consist of suspending the microorganisms in the wastewater. While in the water, they absorb the organic waste and nutrients around them, which allows them to grow and reproduce to form micro-colonies. These micro-colonies settle as sludge, which is then removed and either reused in the process by being re-suspended, or treated in a sludge treatment process. Some examples of suspended film are: activated sludge, extended aeration, and sequential batch reactor systems.
- Lagoon Systems Lagoon systems are settling ponds in which the wastewater is retained for an extended period of time. During this time, microorganisms degrade the organic compounds present in the water

Biofertilizers

- Biofertilizers are substance that contains microbes, which helps in promoting the growth of plants and trees by increasing the supply of essential nutrients to the plants. It comprises living organisms which include mycorrhizal fungi, blue-green algae, and bacteria.
- Symbiotic Nitrogen-Fixing Bacteria
- Loose Association of Nitrogen-Fixing Bacteria
- Symbiotic Nitrogen-Fixing Cyanobacteria
- Free-Living Nitrogen-Fixing Bacteria

Types

- I. N2 fixers

a. Free living :

Aerobic – Azotobacter, Beijerinckia, Anabaena

Anaerobic – Clostridium

Faultative anaerobic – Klebsiella

b. Symbiotic : Rhizobium, Frankia, Anabaena azollae

c. Associative symbiotic : Azospirillum

d. Endophytic : Gluconacetobacter Burkholdria

- II. Phosphorus solubilizers Bacteria : Bacillus megaterium var. phosphaticum B. subtilis, B. circulans Pseudomonas striata Fungi : Penicillium sp. Aspergillus awamori
- III P mobilizers

  a) AM fungi
- b) Ectomycorrhizal fungi
- c) Ericoid Mycorrhiza
- d) Orchid mycorrhiza
- IV. Silicate and Zinc solubilizers: Bacillus sp,
- V. Plant growth promoting Rhizobacteria: Pseudomans spp.,and many more

Importance of Biofertilizers

- Biofertilizers are known to make a number of positive contributions in agriculture.
- Supplement fertilizer supplies for meeting the nutrient needs of crops.
- Add 20 – 200 kg N/ha (by fixation) under optimum conditions and solubilise/mobilise 30-50 kg P_2O_5/ha.
- They liberate growth promoting substances and vitamins and help to maintain soil fertility.
- They suppress the incidence of pathogens and control diseases.
- Increase the crop yield by 10-50%. N_2 fixers reduce depletion of soil nutrients and provide sustainability to the farming system.
- Cheaper, pollution free and based on renewable energy sources.
- They improve soil physical properties, tilth and soil health.

Refer attached document.

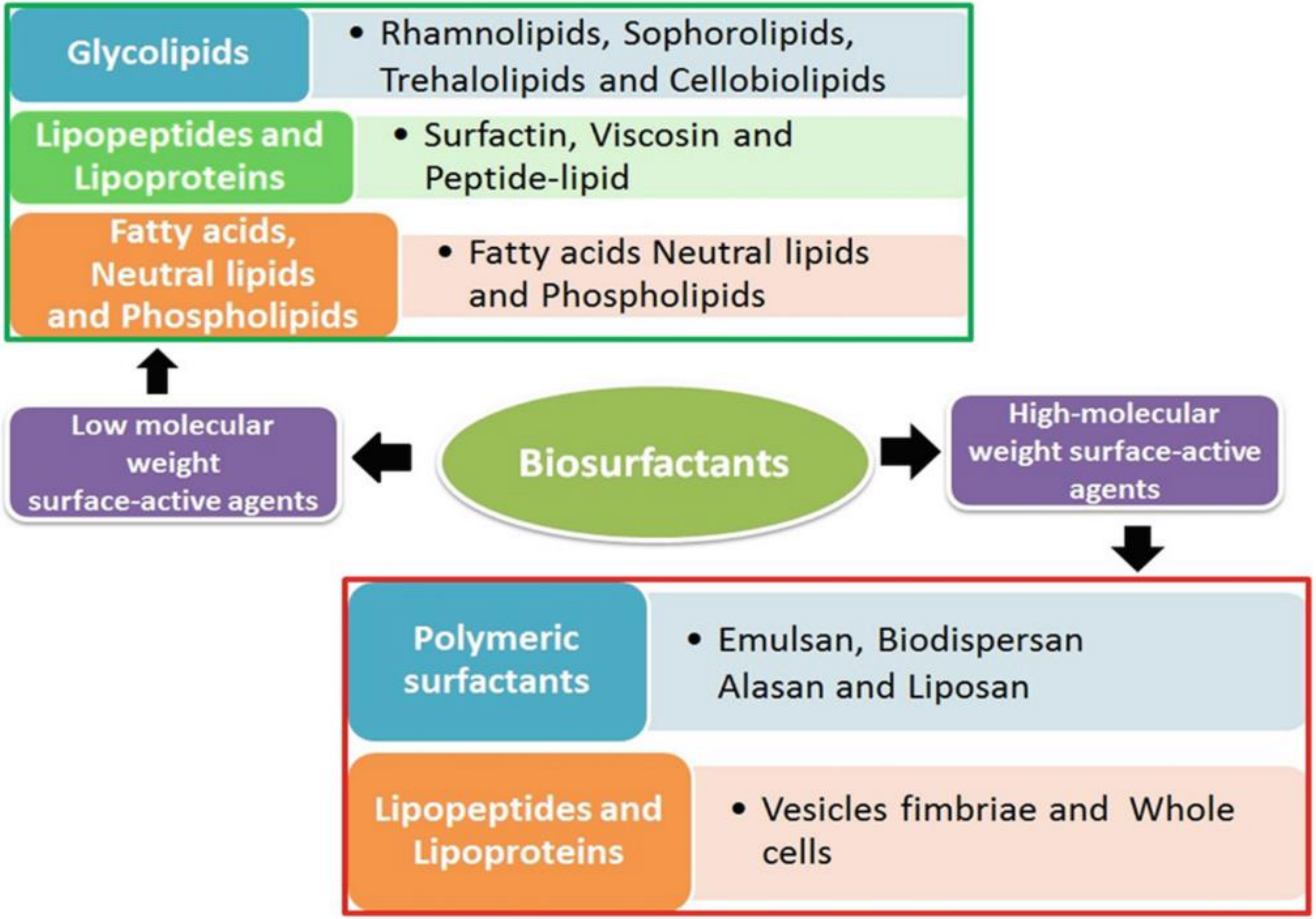
Biosurfactants

Biosurfactants are active compounds that are produced at the microbial cell surface or excreted, and reduce surface and interfacial tension. Microbial surfactants offer several advantages over synthetic ones, such as low toxicity and high biodegradability, and remain active at extreme pH and salinity.

Biosurfactants are produced by bacteria, yeasts, and filamentous fungi.

Types

- Biosurfactants with lower molecular weight (e.g., glycolipids and low-molecular-weight lipopeptides (LPs) and phospholipids) show industrial potential because of their capacity in reducing surface and interfacial tension. Among glycolipids, those that have a greater interest are trehalolipids, cellobiose lipids, mannosylerythritol lipids, rhamnolipids (derived mostly from *Pseudomonas*), and sophorolipids (SLs) (derived from *Candida* and related species).
- Besides, the potential therapeutic uses attributed to these biosurfactants with high molecular weight (such as polysaccharides, lipoproteins, or lipopolysaccharides) include their surface adherence and emulsifier properties



Biosurfactants

Low molecular weight surface-active agents

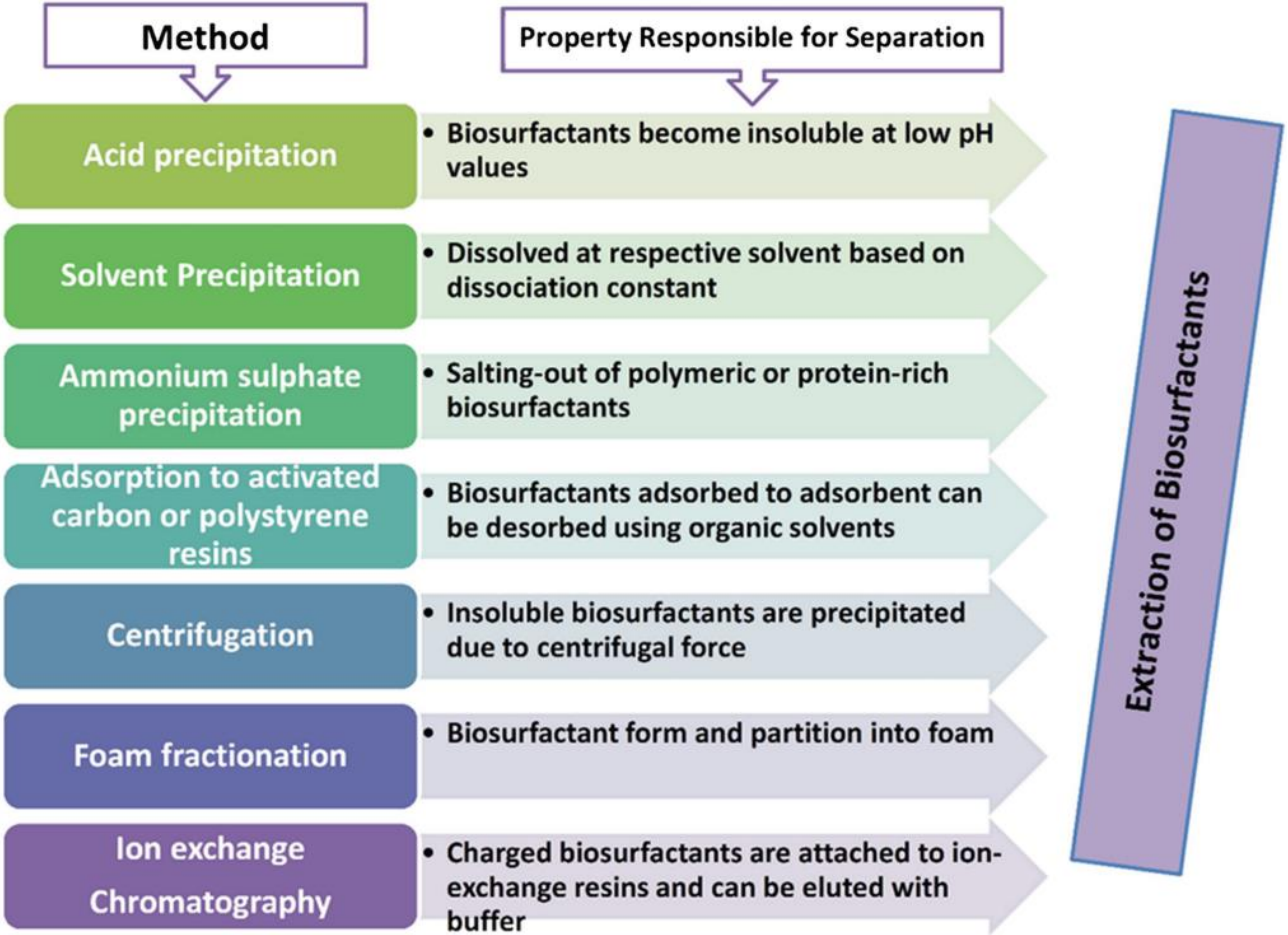
Glycolipids	Rhamnolipids, Sophorolipids, Trehalolipids and Cellobiolipids
Lipopeptides and Lipoproteins	Surfactin, Viscosin and Peptide-lipid
Fatty acids, Neutral lipids and Phospholipids	Fatty acids Neutral lipids and Phospholipids

High-molecular weight surface-active agents

Polymeric surfactants	Emulsan, Biodispersan Alasan and Liposan
Lipopeptides and Lipoproteins	Vesicles fimbriae and Whole cells

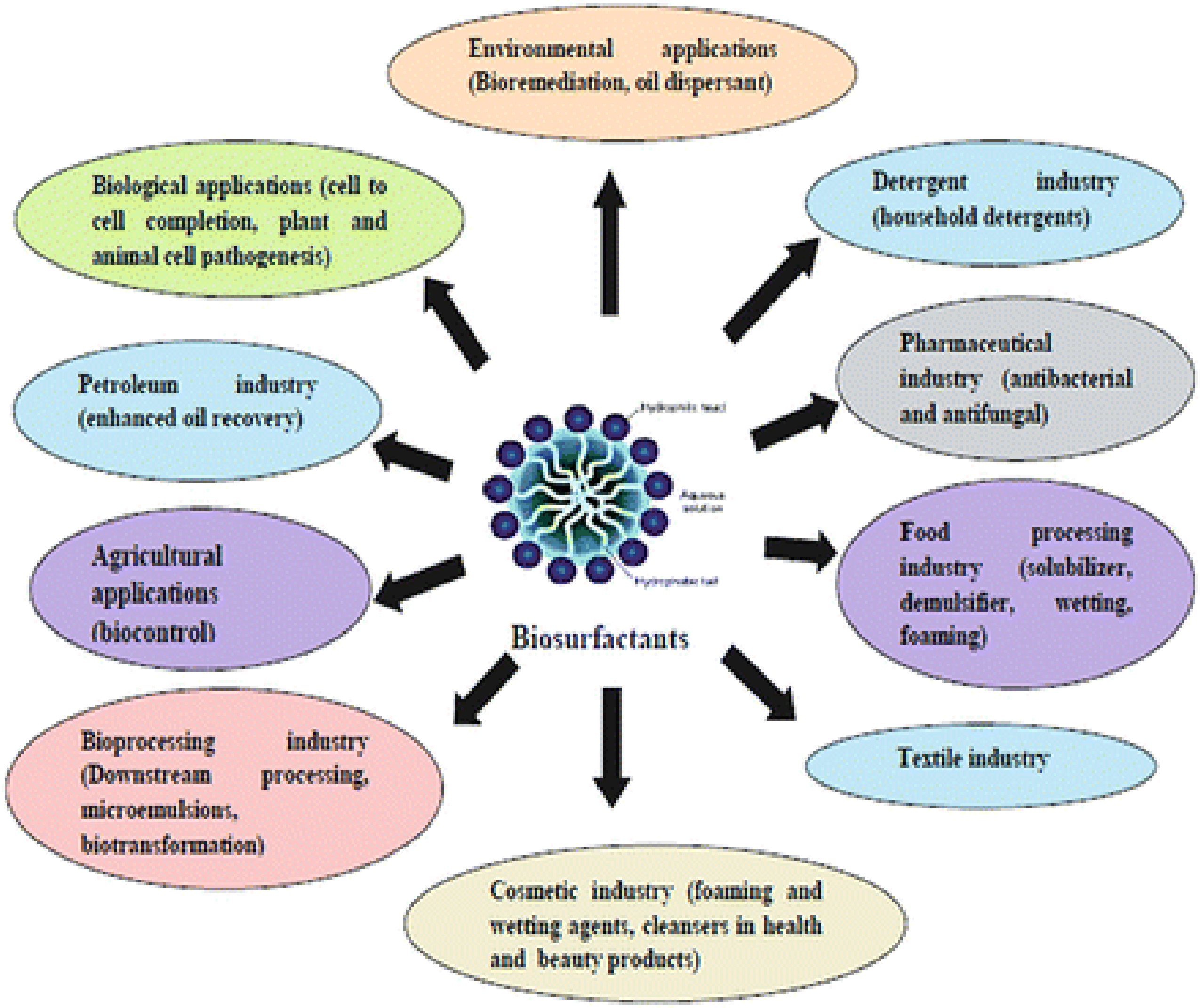
Biosurfactant	Microbial source	References
Rhamnolipids	*Pseudomonas aeruginosa*	[18]
	Pseudomonas chlororaphis	
	Serratia rubidea	
Sophorolipids	*Candida bombicola*	[5]
	Candida batistae	[20]
	Trichosporon ashii	[6]
Saphorose Lipid	*Torulopsis bombicola*	[21]
Trehalose lipids	*Rhodococcus erythropolis*	[22]
	Arthrobacter Sp.	
	Nocardia erythropolis	
	Corneybacterium sp.	
	Mycobacterium sp	
Ornithine lipids	*Pseudomonas sp*	[11]
	Thiobacillus thiooxidans	
	Agrobacterium sp	
Viscosin	*Pseudomonas fluoresens*	[3]
	Leuconostoc mesenteroids	
Carbohydrate lipid	*Pseudomonas fluoresens*	[25]
	Debaryomyces polmorphus	

Table 1: Microbial source and type of biosurfactants.



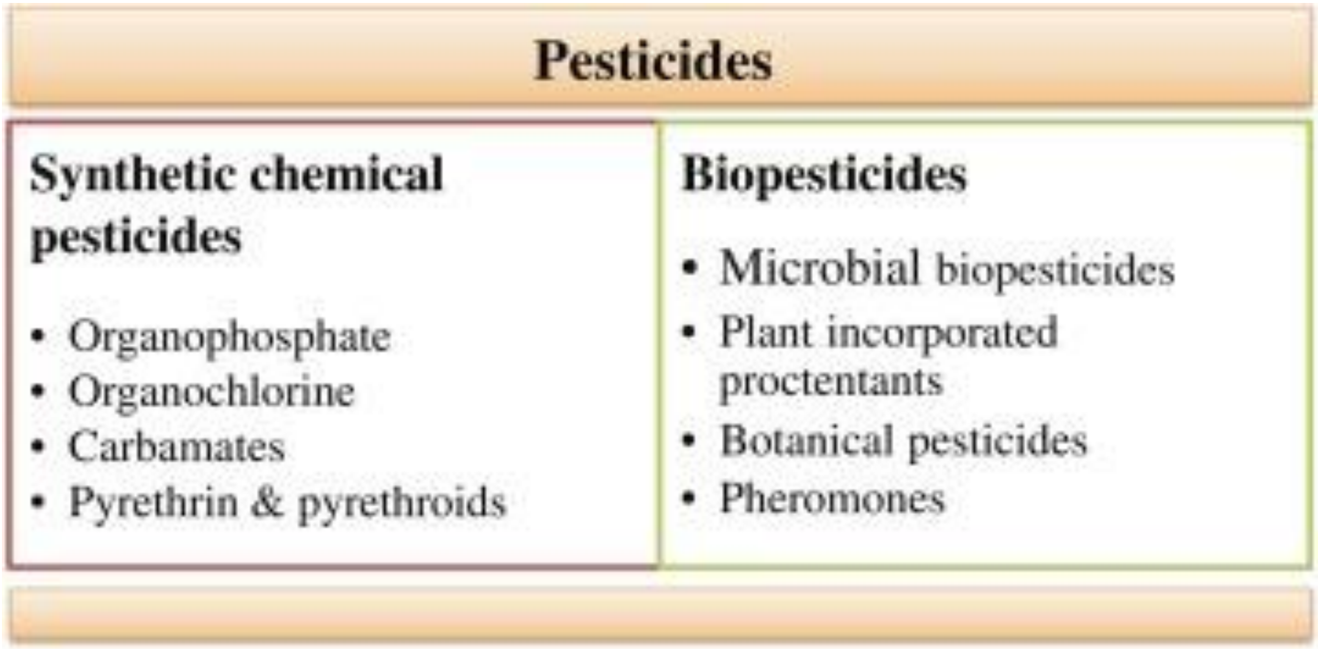
Method	Property Responsible for Separation
Acid precipitation	Biosurfactants become insoluble at low pH values
Solvent Precipitation	Dissolved at respective solvent based on dissociation constant
Ammonium sulphate precipitation	Salting-out of polymeric or protein-rich biosurfactants
Adsorption to activated carbon or polystyrene resins	Biosurfactants adsorbed to adsorbent can be desorbed using organic solvents
Centrifugation	Insoluble biosurfactants are precipitated due to centrifugal force
Foam fractionation	Biosurfactant form and partition into foam
Ion exchange Chromatography	Charged biosurfactants are attached to ion-exchange resins and can be eluted with buffer

Extraction of Biosurfactants


Environmental applications (Bioremediation, oil dispersant)
Biological applications (cell to cell completion, plant and animal cell pathogenesis)
Detergent industry (household detergents)
Petroleum industry (enhanced oil recovery)
Pharmaceutical industry (antibacterial and antifungal)
Hydrophilic head
Aqueous solution
Hydrophobic tail
Agricultural applications (biocontrol)
Food processing industry (solubilizer, demulsifier, wetting, foaming)
Biosurfactants
Textile industry
Bioprocessing industry (Downstream processing, microemulsions, biotransformation)
Cosmetic industry (foaming and wetting agents, cleansers in health and beauty products)

Biopesticides

- Biopesticides are types of chemicals extracted from natural materials such as plants, animals, bacteria or certain minerals and these chemicals can be used for controlling pests. For example, canola oil/baking soda with pesticidal applications are considered biopesticides.



Pesticides

Synthetic chemical pesticides	Biopesticides
Organophosphate Organochlorine Carbamates Pyrethrin & pyrethroids	Microbial biopesticides Plant incorporated proctentants Botanical pesticides Pheromones

- Biopesticides can be considered as dividing into three major classes
- 1. **Microbial pesticides** consist of microorganism (e.g. bacterium, fungus, virus or protozoan) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest(s). For example, there are fungi that can control certain weeds, and other fungi that can kill specific insects.
- 2**. Biochemical pesticides** are naturally occurring substances that control pests by non-toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest. Biochemical pesticides include substances, such as insect sex pheromones, which interfere with mating, as well as various scented plant extracts that attract insect pests to traps. Because it is sometimes difficult to determine whether a substance meets the criteria for classification as a biochemical pesticide, responsible authority would establish a special committee to make such decisions.
- 3. **Plant-Incorporated-Protectants** (PIPs) are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can take the gene for the B.t. pesticidal protein, and introduce the gene into the plant's own genetic material. Then the plant, instead of the B.t. bacterium, manufactures the substance that destroys the pest

Microbial biopesticides	Efficacy
***Trichoderma* spp.**	**Antagonistic fungal agents used widely for controlling several pests and as plant growth enhancers. It is also used for production of commercially important enzymes-hemicellulases, proteases, 1,3-glucanase and cellulases**
Pseudomonas fluorescens	This bacteria controls root rot and wilts diseases of banana, bean, cotton, groundnut, pigeon pea, soya and tomato. It is also effective against s the rice blast and sheath blight of paddy.
Beauveria bassiana	It is infectious against 200 insect hosts and can also be used in the management of various species of termites, grasshoppers, pyrilla of sugarcane, root grub, coconut *Rhinoceros* beetle brown plant hopper (BPH) in paddy and coffee berry borer
Lecanicillium lecanii	It is used against soft bodies, sucking type pests, present in green house conditions. It is highly effective against thrips, aphids, hoppers, scales, mites and whiteflies.
Nomuraea rileyi	It acts as a natural mortality factor in some major lepidopteran insect pests like *Helicoverpa* sp., Semiloopers, *Spodoptera* sp., cutworms in transitional climates with high humidity.
Hirsutella thompsonii	A pathogenic fungus which causes infection in mites present on crops, fruits and plantation crops. It can be easily mass produced on carbon rich substratum particularly rice and sorghum. It is facultative in their nature.

Formulation

- Formulation refers to the preparation of a product from an active ingredient by the addition of certain active (functional) and non-active(inert) substances (Grewal, 2005).
- Biopesticides are usually formulated as: dry formulations for direct application – dusts (DP), seed dressing formulations – powders for seed dressing (DS), granules (GR), micro granules (MG), dry formulations for dilution in water – water dispersible granules (WG), and wettable powders (WP); liquid formulations for dilution in water – emulsions, suspension concentrates (SC), oil dispersions (OD), suspo-emulsions (SE), capsule suspensions (CS); ultra low volume

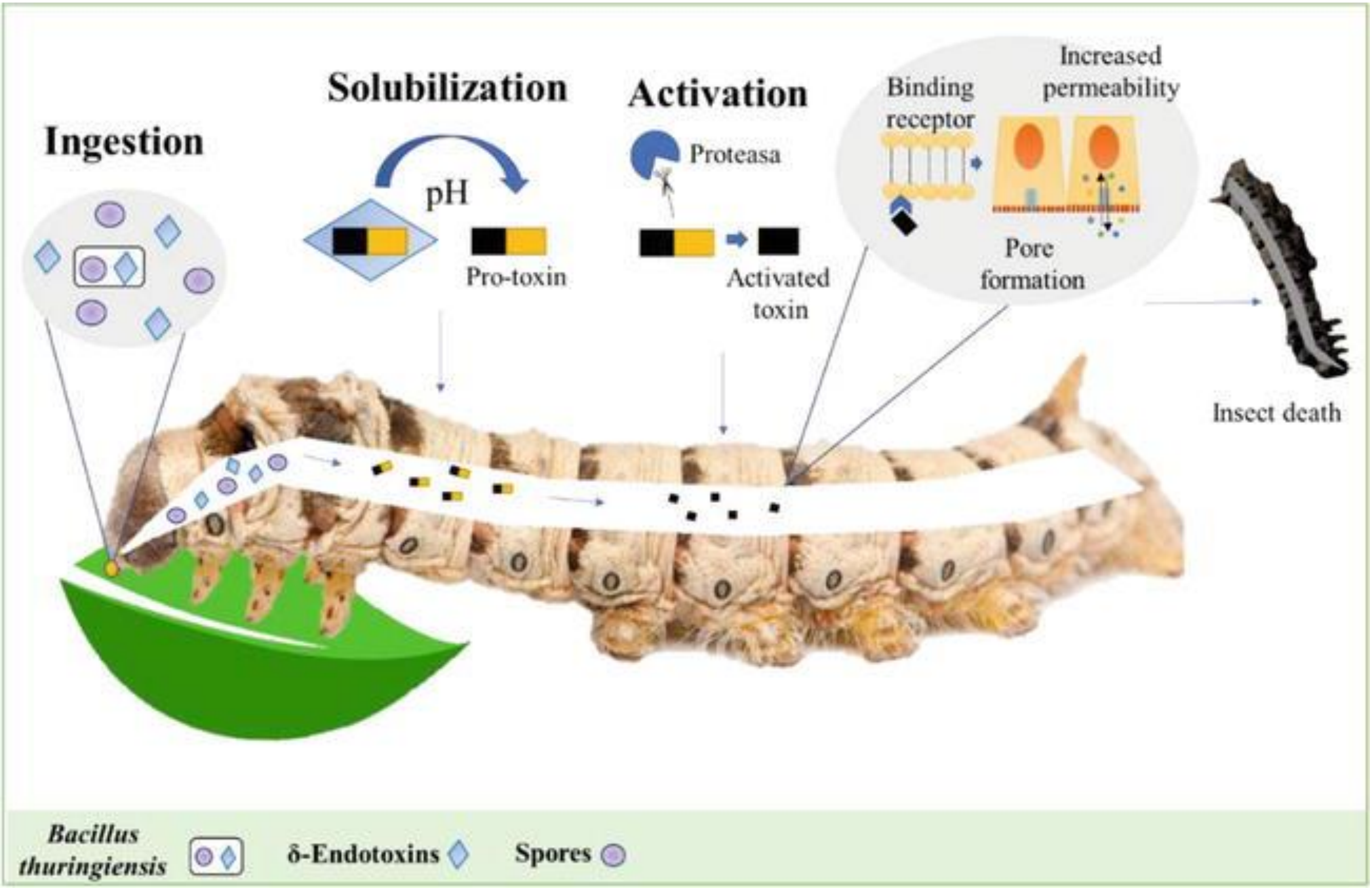
BT Toxins

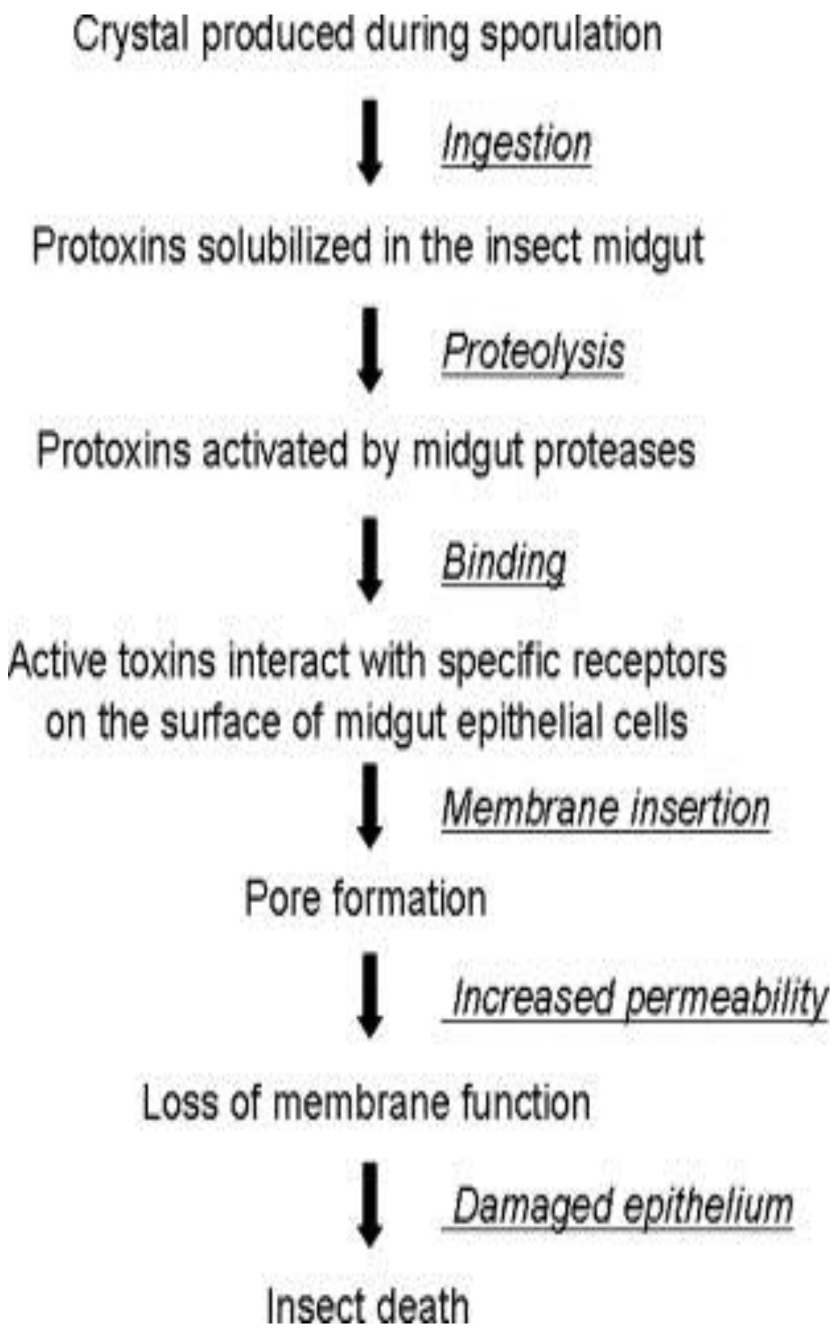
- *BT* is an aerobic, sporeforming, Gram-positive, rod-shaped bacterium microbe naturally found in soil. It makes proteins that are toxic to immature insects (larvae). There are **many types of *Bt***. Each targets different insect groups. Target insects include beetles, **mosquitoes**, black flies, caterpillars, and moths.

- The *Bacillus thuringiensis* δ-endotoxins (Bt toxins) are widely used insecticidal proteins in engineered crops that provide agricultural, economic, and environmental benefits.

- Different strains of Bt strains effective against lepidopteran insects to particular types of depending on the specific crystalline protein (delta-endotoxin) that they produce. For example, Bt *aizawai* (Bta) is effective against wax moth larvae; Bt *israelensis* (Bti) is effective against mosquitoes, blackflies, and some midges; Bt *kurstaki* (Btk) controls various types of lepidopterous species including the gypsy moth and cabbage looper; and Bt *san diego* is effective against certain beetle species and the boll weevil.

Mode of action




Crystal produced during sporulation

↓ *Ingestion*

Protoxins solubilized in the insect midgut

↓ *Proteolysis*

Protoxins activated by midgut proteases

↓ *Binding*

Active toxins interact with specific receptors on the surface of midgut epithelial cells

↓ *Membrane insertion*

Pore formation

↓ *Increased permeability*

Loss of membrane function

↓ *Damaged epithelium*

Insect death

Production

- Mass production of Btk, Bti, Btt, and Bta occurs through either solid or liquid fermentation. The cost of medium alone for the production of a liter of Bti is about $1.2 and $0.01 (United States) using commercial complex medium versus by-products of industrial factories respectively.
- At the completion of fermentation the active ingredient (AI) is either the ICP with a spore mix or an enriched ICP harvested through centrifugation, filtration, or preparative precipitation. Subsequent AI is mixed with carriers (lignocellulosic, starch, or earth material) for dry dust application or in concentrated emulsion (water in oil) for land or aquatic application. Often sunscreen (PABA) is included in the formulation. The efficacy of the Bt as a bacterial insecticide (BI) is then standardized and sold.

Nuclear Polyhedrosis Virus

- The nuclear polyhedrosis virus (NPV), part of the family of baculoviruses, is a virus affecting insects, predominantly moths and butterflies. It has been used as a pesticide.

Single cell protein

- refer pdf attached.

Single cell oil

- Single cell oils (SCOs) are intracellular storage lipids comprising of triacyglycerols (TAGs). SCOs are produced by oleaginous microorganisms which are able to accumulate between 20% and up to 80% lipid per dry biomass in the stationary growth phase under nutrient limitations, e.g., nitrogen or phosphor, with simultaneous excess of carbon source.
- The fermentative production can be realized by submerged (SmF) or solid state fermentation (SSF). The yield and the composition of the obtained microbial lipids depend on the type of fermentation and the particular conditions (e.g., medium, pH-value, temperature, aeration, nitrogen source).

Lipid formation inside microbial cell

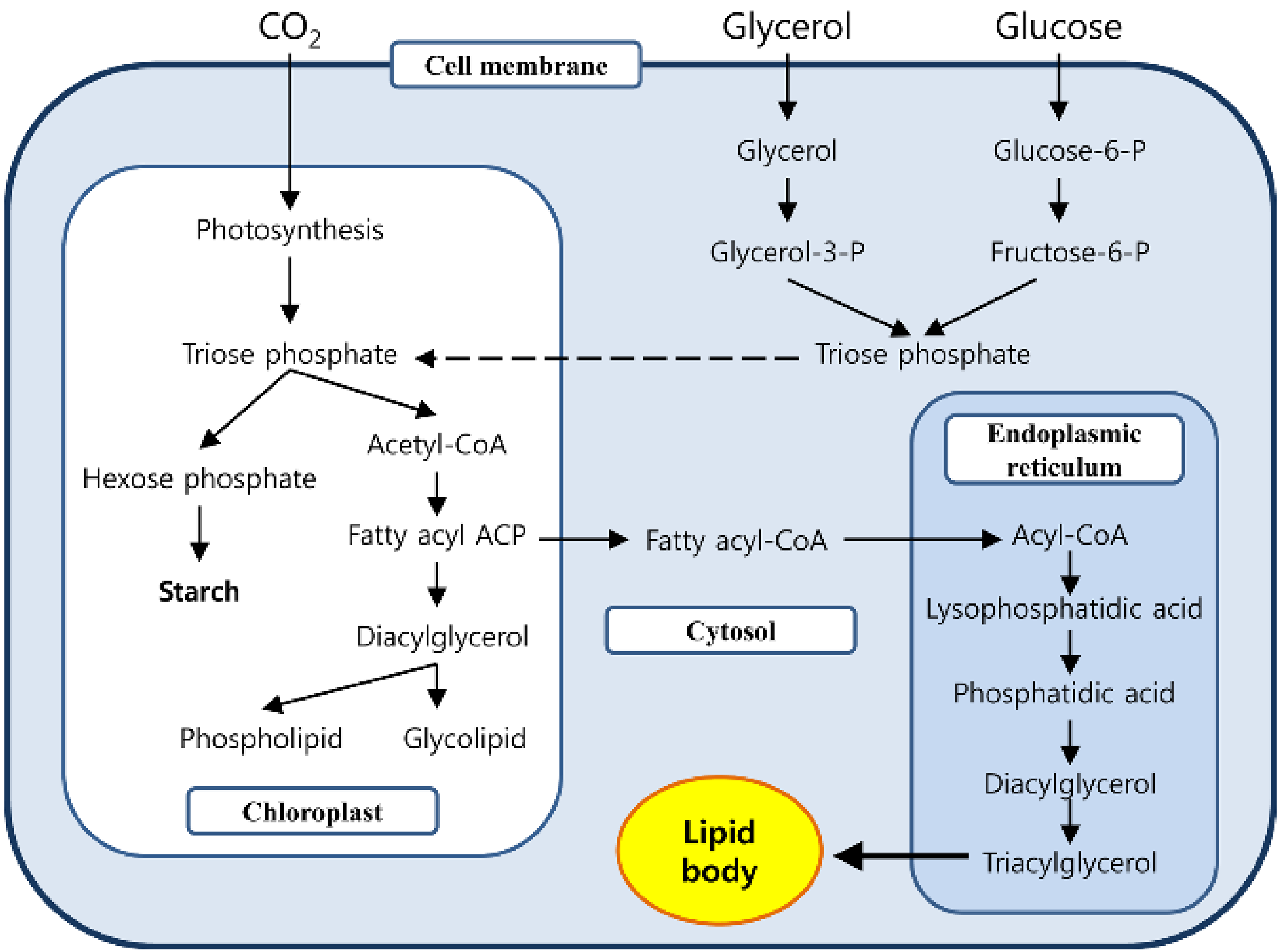


Fig. 3 Carbon capture and lipid biosynthesis pathway in

Physiology of Microbial Oil Production

Microorganisms produce and accumulate oils as an energy reserve. When the microorganisms are grown in a medium in which carbon is plentiful but another essential nutrient is scarce, the cells rapidly grow and proliferate until the limited nutrient is exhausted. Then the microbes [b]stop dividing but continue to grow. Usually, in industrial fermentations nitrogen is the growth limiting nutrient. When all the nitrogen in the growth medium is used up, the cells cannot create new cells since the protein and nucleic acid synthesis is stopped. But they continue to assimilate carbon and convert it into oils and fats.

The amount and the nature of the accumulated lipids depend on the type of microorganism and are genetically predetermined. ATP citrate lyase[b] is the enzyme that is responsible for lipid accumulation in larger quantities. The presence of this enzyme implies that the microorganism can stock lipids more than 20% their biomass.

Microorganisms for SCO Production

- High amounts of cellular lipids are produced by microorganisms belonging to the genera *Cryptococcus, Cunninghamella*, and *Mortierella*.
- The genus *Mortierella* is capable to produce SCO with a unique composition, containing high amounts of PUFAs (Asadi et al., 2015).
- *M. alpina* is used in an industrial process for the production of arachidonic acid (ARA, 20:4, ω-6) for food supplementation by DSM (Béligon et al., 2016).
- The most frequently used carbon source is glucose. Various mono- or disaccharides and carboxymethyl-cellulose (CMC) were tested as carbon source for *Mortierella isabellina* by Zeng et al. (2013). In this study cellular lipid contents above 60% were generated with xylose, glucose and fructose as substrates. CMC was a poor substrate, implying the absence of a cellulase system.

Downstream processing

- Downstream processing costs are one of the major obstacles to be solved for full economic efficiency of microbial lipids. Because single cell oils are formed intracellular for storage purposes, they have to be extracted upon further applications as long as production strains are not engineered to excrete TAGs or free fatty acids which would drastically simplify downstream processing.
- Cell disruption methods have the potential to enhance oil recovery yields (Koubaa et al., 2020). The most investigated technologies include bead milling, high-pressure homogenization (HPH), ultrasound- and microwave-assisted extraction (UAE and MAE, respectively).

Examples of oils

- **Linolenic acid:**
- Single-cell oil rich in linolenic acid was the first oil produced by the fungus *Mucor circinelloides.* Its production however, was later discontinued due to cost factor.
- **Arachidonic acid:**
- The soil fungus, *Mortierella alpina* has been used for the production of oils rich in arachidonic acid.
- **Docosahexaenoic acid:**
- The marine alga, *Crypthecodinium cohnii* has been employed for commercial production of decosahexaenoic acid (DHA). Microbial DHA is used for inclusion, in baby foods.

Industrial Production of Single Cell Oils

- In commercial manufacturing plants, oil rich biomass is produced by **fermentation** of sugar rich media.
- These cells are then **seperated** by centrifugation. Then the oils are **extracted** by disrupting the cell walls in the presence of a solvent such as hexane and evaporating the solvent by drying under vacuum conditions.
- Finally, the extracted crude oil is **refined** through a series of steps including neutralisation, degumming, bleaching and deodorisation.

Single Cell Oils in the Market

An infant formula including a blend of single cell oils, namely arachidonic acid (ARA) and docosahexaenoic acid (DHA) is currently available in Europe, Australasia, Far East and USA. In addition, cocoa butter-like products are produced using several species of yeasts such as *Cryptococcus curvatus*.

- Furthermore, many polyunsaturated fatty acids (PUFA) which have gained interest as dietary supplements and nutraceuticles, are being synthesised using microorganisms, mostly fungal and algal species.

Biofuels

Biofuel is any kind of fuel that is directly derived from plant or animal matter, also known as biomass, and produces bioenergy. ‘Bio’ is used to depict the organic nature of this fuel source because it is not produced by a geological process like that of fossil fuels (petroleum and coal).

4 biofuel sources,

Algae

- Algae come from stagnant ponds in the natural world, and more recently in algae farms, which produce the plant for the specific purpose of creating biofuel. Advantage of algae focus on the followings:
- No CO2 back into the air, self-generating biomass, Algae can produce up to 300 times more oil per acre than conventional crops. Among other uses, algae have been used experimentally as a new form of green jet fuel designed for commercial travel. At the moment, the upfront costs of producing biofuel from algae on a mass scale are in process, but are not yet commercially viable.

2.2. Carbohydrate

- (sugars) rich biomaterial It comes from the fermentation of starches derived from agricultural products like corn, sugar cane, wheat, beets, and other existing food crops, or from inedible cellulose from the same. Produced from existing crops, can be used in an existing gasoline engine, making it a logical transition from petroleum. It used in Auto industry, heating buildings ("flueless fireplaces") At present, the transportation costs required to transport grains from harvesting to process- ing, and then out to vendors results in a very small net gain in the sustainability stakes.

2.3. Oils rich biomaterial

- It comes from existing food crops like rapeseed (aka Canola), sunflower, corn, and others, af- ter it has been used for other purposes, i.e food preparation (“waste vegetable oil”, or WVO), or even in first use form (“straight vegetable oil”, or SVO). Not susceptible to micro- bial degradation, high availability, re-used material. It is used in the creation of biodiesel fuel for automobiles, home heating, and experimentally as a pure fuel itself. At present, WVO or SVO is not recognized as a mainstream fuel for automobiles. Also, WVO and SVO are susceptible to low temperatures, making them unusable in colder climates.

2.4. Agriculture wastes

- (organic and inorganic sources) It comes from agricultural waste which is concentrated into charcoal-like biomass by heat- ing it. Very little processing required, low-tech, naturally holds CO2 rather than releasing it into the air. Primarily, biochar has been used as a means to enrich soil by keeping CO2 in it, and not into the air. As fuel, the off-gasses have been used in home heating. There is contro- versy surrounding the amount of acreage it would take to make fuel production based on biochar viable on a meaningful scale. Furthermore, use of agriculture wastes which rich with inorganic elements (NPK----) as compost (fertilizer) in agriculture.

Types

- Wood. This is the most basic form of fuel that is derived from organic matter. ...
- Biogas. This is the gaseous form of biofuels. ...
- Biodiesel. This biofuel is liquid in nature. ...
- Ethanol. ...
- Methanol. ...
- Butanol.

Biodiesel

- Biodiesel is a clean-burning diesel fuel produced from vegetable oils, animal fats, or grease. Its chemical structure is that of fatty acid alkyl esters (FAAE). Biodiesel as a fuel gives much lower toxic air emissions than fossil diesel. In addition, it gives cleaner burning and has less sulfur content, and thus reducing emissions.
- Commercially, biodiesel is produced by transes- terification of triglycerides which are the main ingredients of biological origin oils in the presence of an alcohol (e.g. methanol, ethanol) and a catalyst (e.g. alkali, acid, enzyme) with glycerine as a major by-product.
- Blends with diesel fuel are indicated as ‘‘Bx’’, where ‘‘x’’ is the percentage of biodiesel in the blend. For instance, ‘‘B5’’ indicates a blend with 5% biodiesel and 95% diesel fuel; in consequence, B100 indicates pure biodiesel.

Production

- Biodiesel is produced from vegetable oils, yellow grease, used cooking oils, or animal fats. The fuel is produced by **transesterification**—a process that converts fats and oils into biodiesel and glycerin (a coproduct). Approximately 100 pounds of oil or fat are reacted with 10 pounds of a short-chain alcohol (usually methanol) in the presence of a catalyst (usually sodium hydroxide [NaOH] or potassium hydroxide [KOH]) to form 100 pounds of biodiesel and 10 pounds of glycerin (or glycerol). Glycerin, a co-product, is a sugar commonly used in the manufacture of pharmaceuticals and cosmetics.

Transesterification

- This chemical reaction converts an ester (vegetable oil or animal fat) into a mixture of esters of the fatty acids that makes up the oil (or fat).
- Biodiesel is obtained from the purification of the mixture of fatty acid methyl esters (FAME).
- A catalyst is used to accelerate the reaction . According to the catalyst used, transesterification can be basic, acidic or enzymatic, the former being the most frequently used.

- Although transesterification is the most important step in biodiesel production (since it originates the mixture of esters), additional steps are necessary to obtain a product that complies with international standards.
- **Stages of Biodiesel Production Process**

➢ Treatment of raw materials

➢ Alcohol-catalyst mixing

➢ Chemical reaction

➢ Separation of the reaction products

➢ Purification of the reaction products

1. Treatment of Raw Materials

The content of free fatty acids, water and non-saponificable substances are key parameters to achieve high conversion efficiency in the transesterification reaction.

2. Alcohol-Catalyst Mixing

The alcohol used for biodiesel production must be mixed with the catalyst before adding the oil. The mixture is stirred until the catalyst is completely dissolved in the alcohol. It must be noted that the alcohol must be water-free (anhydrous).

Sodium and potassium hydroxides are among the most widely used basic catalysts. For production on an industrial scale, sodium or potassium methoxides or methylates are commercially available.

3. Chemical Reaction

- The chemical reaction takes place when the oil is mixed with the alkoxide (alcohol–catalyst mix).This requires certain conditions [17, 21] of time, temperature and stirring. Since alcohols and oils do not mix at room temperature, the chemical reaction is usually carried out at a higher temperature and under continuous stirring, to increase the mass transfer between the phases.

4. Catalysts

The catalysts used for the transesterification of triglycerides may be classified as basic, acid or enzymatic,

- Basic catalysts include sodium hydroxide (NaOH), potassium hydroxide (KOH), carbonates and their corresponding alcoxides (for instance, sodium methoxide or ethoxide
- Acid catalysts include sulfuric acid, sulfonic acids and hydrochloric acid; their use has been less studied
- Heterogeneous catalysts that have been considered for biodiesel production include enzymes [39], titanium silicates [58], and compounds from alkaline earth metals [59], anion exchange resins [59] and guanidines in organic polymers [60]. Lipases are the most frequently used enzymes for biodiesel production

- 5. Separation of the Reaction Products
- The separation of reaction products takes place by decantation: the mixture of fatty acids methyl esters (FAME) separates from glycerin forming two phases, since they have different densities; the two phases begin to form immediately after the stirring of the mixture is stopped.
- Thin Layer Chromatography [26]
- Gas Chromatography (GC) [27–29]
- High Performance Liquid Chromatography (HPLC) [29–33] Gel Permeation Chromatography [34]
- Nuclear Magnetic Resonance (NMR) [35, 36]
- Infrared (IR) Spectroscopy

6. Purification of the Reaction Products

The mixture of fatty acids methyl esters (FAME) obtained from the transesterification reaction must be purified in order to comply with established quality standards for biodiesel.

Therefore, FAME must be washed, neutralized and dried. Successive washing steps with water remove the remains of methanol, catalyst and glycerin, since these contaminants are water-soluble.

- The first washing step is carried out with acidified water, to neutralize the mixture of esters.
- Then, two additional washing steps are made with water only.
- Finally the traces of water must be eliminated by a drying step.
- After drying, the purified product is ready for characterization as biodiesel according to international standards.

Raw Materials for Biodiesel Production

- The raw materials for biodiesel production are vegetable oils, animal fats and short chain alcohols. The oils most used for worldwide biodiesel production are
- rapeseed (mainly in the European Union countries),
- soybean (Argentina and the United States of America),
- palm (Asian and Central American countries) and
- sunflower, although other oils are also used,
- including peanut, linseed, safflower, used vegetable oils, and also animal fats. Methanol is the most frequently used alcohol although ethanol can also be used.

Microalgae

- Microalgae have great potential for biodiesel production, since the oil yield (in liters per hectare) could be one to two orders of magnitude higher than that of other raw materials. Oil content is usually from 20 to 50%, although in some species it can be higher than 70% [13]. However, it is important to note that not all microalgae are adequate for biodiesel production. High levels of CO2, water, light, nutrients and mineral salts are necessary for the growth of microalgae. Production processes take place in raceway ponds and photobiological reactors

Advantages of the Use of Biodiesel

- • Renewable fuel, obtained from vegetable oils or animal fats.
- • Low toxicity, in comparison with diesel fuel.
- • Degrades more rapidly than diesel fuel, minimizing the environmental consequences of biofuel spills.
- • Lower emissions of contaminants: carbon monoxide, particulate matter, polycyclic aromatic hydrocarbons, aldehydes.
- • Lower health risk, due to reduced emissions of carcinogenic substances.
- • No sulfur dioxide (SO2) emissions.
- • Higher flash point (100C minimum).

- • May be blended with diesel fuel at any proportion; both fuels may be mixed during the fuel supply to vehicles.
- • Excellent properties as a lubricant.
- • It is the only alternative fuel that can be used in a conventional diesel engine, without modifications.
- • Used cooking oils and fat residues from meat processing may be used as raw materials

Bio hydrogen

- Biohydrogen production technology is the process of catalyzing hydrogen production by microorganisms through light energy or fermentation and taking organic compounds in nature as substrate at normal temperature and in a normal-pressure aqueous solution.

Production

- At present hydrogen is produced mainly from fossil fuels, biomass and water.
- The methods of hydrogen production from fossil fuels are

(a) Steam reforming of natural gas.

(b) Thermal cracking of natural gas.

(c) Partial oxidation of heavier than naphtha hydrocarbons.

(d) Coal gassification.

2. Methods of hydrogen production from biomass are

- (e) Pyrolysis or gassification (which produces a mixture of gases, i.e., H_2; CH_4; CO_2; CO; N_2).

3. Methods of hydrogen production from water are

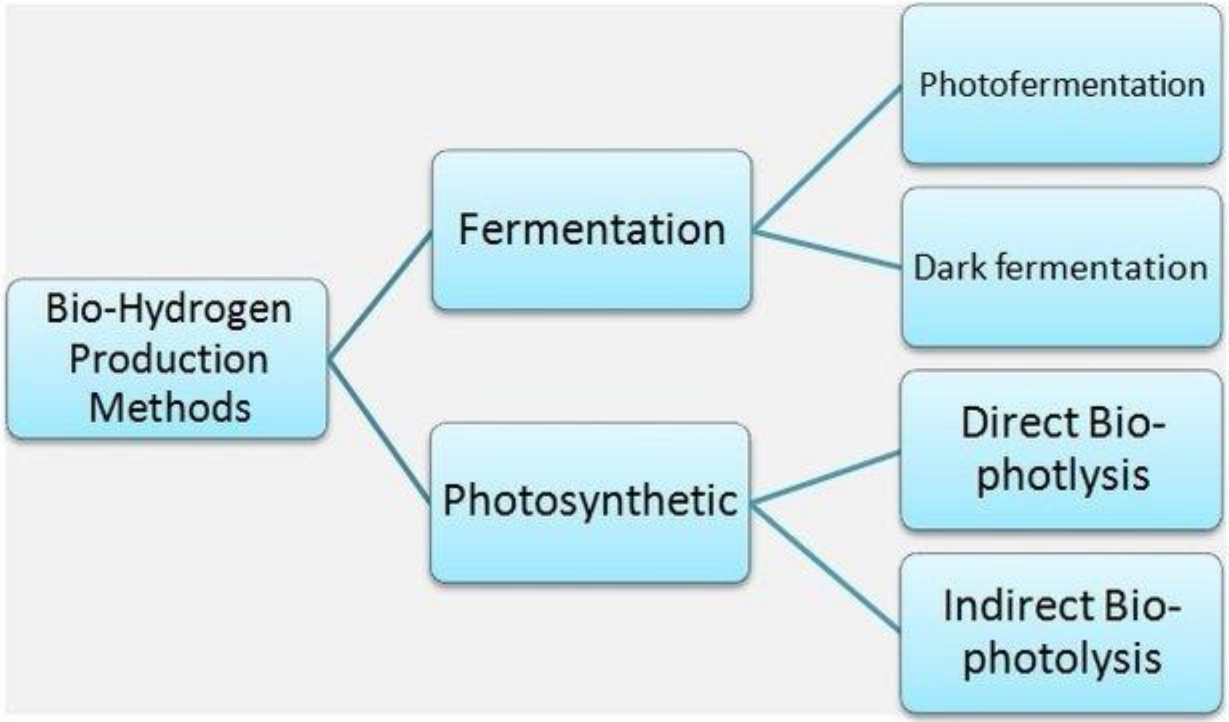
- (f) Electrolysis.
- (g) Photolysis.
- (h) Thermochemical process.
- (i) Direct thermal decomposition or thermolysis.
- (j) Biological production.

Biological Hydrogen Production Methods

- Biological hydrogen production methods can be classificated as below:
- 2.1. Direct biophotolysis
- 2.2. Indirect biophotolysis
- 2.3. Photo fermentation
- 2.4. Dark fermentation
- 2.5. Two stage process

(integration of dark and photo fermentation)

- 2.6. Biocatalyzed electrolysis



a.Direct biophotolysis

- This method is similar to the processes found in plants and algal photosynthesis. In this process solar energy is directly converted to hydrogen via photosynthetic reactions

$2H_2O$ + 'light energy'→ $2H_2 + O_2$.

- Algae split water molecules to hydrogen ion and oxygen via photosynthesis.
- The generated hydrogen ions are converted into hydrogen gas by hydrogenase enzyme.
- *Chlamydomonas reinhardtii* is one of the well-known hydrogen producing algae [4]. Hydrogenase activity has also been observed in other green algae like *Scenedesmus obliquus, Chlorococcum littorale, Platymonas subcordiformis and Chlorella fusca* [2].
- The advantage of this method is that the primary feed is water, which is inexpensive and available almost everywhere

b.Dark fermentation

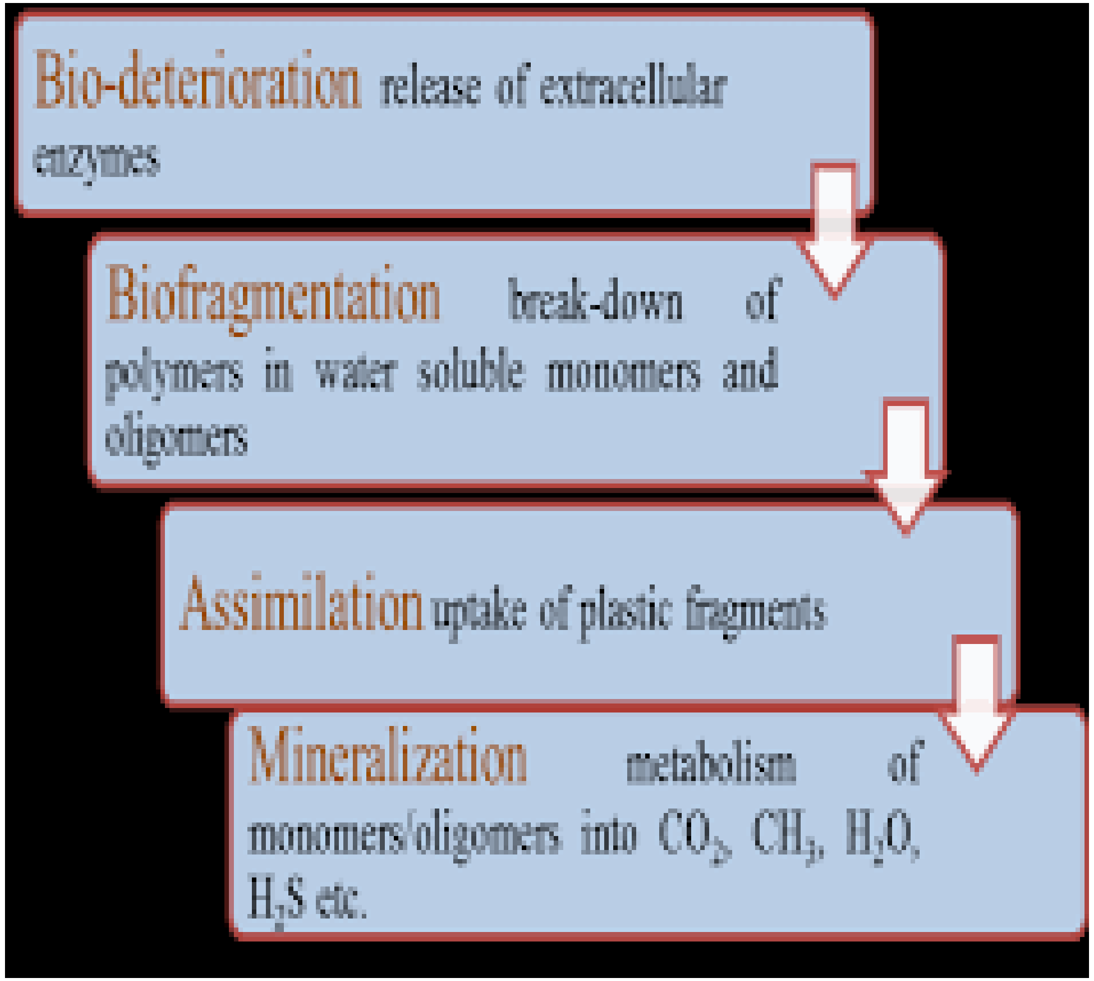
- . Hydrogen can be produced by anaerobic bacteria, grown in the dark on carbohydrate-rich substrates.
- Bacteria known to produce hydrogen include species of Enterobacter, Bacillus, and Clostridium [6].
- Carbohydrates, mainly glucose, are the preferred carbon sources for fermentation processes, which predominantly give rise to acetic and butyric acids together with hydrogen gas [25].
- Theoretically bioconversion of 1 mol of glucose yields 12 mol of hydrogen gas (H2).
- According to reaction stoichiometry, bioconversion of 1 mol of glucose into acetate yields 4 mol H2/mol glucose (Eq. (6)), but only 2 molH2/mol glucose is formed when butyrate is the end product (Eq. (7)) [4]. Currently fermentative processes produce 2.4 to 3.2 moles of hydrogen per mole glucose

Microbial biodegradation

- Biodegradation is the action of microorganisms to decompose a substance into its constituent elements or new compounds.
- Bacteria and fungi have very diverse metabolisms: they use a wide variety of food and energy sources, and perform many important functions. One especially important function is decomposition. Decomposers are bacteria and fungi that can break down organic matter and in doing so recycle nutrients. The most efficient decomposers are those that use aerobic respiration, using oxygen in the process of decomposition.

Process

- The process of biodegradation can be divided into three stages:
- **biodeterioration, biofragmentation, and assimilation**.
- A very broad sense, in nature, there is no waste because almost everything
- gets recycled. In the microbiological sense, “biodegradation” means the
- decaying of all organic materials that is carried out by life forms comprising
- mainly bacteria, fungi, protozoa and other organisms.



Bio-deterioration release of extracellular enzymes

Biofragmentation break-down of polymers in water soluble monomers and oligomers

Assimilation uptake of plastic fragments

Mineralization metabolism of monomers/oligomers into CO_2, CH_4, H_2O, H_2S etc.

- Depending on the nature of the micro-organisms, they may eat a part of the organic molecule only, destroying the intact parent substance in a process known as “primary biodegradation”, or they may eat it completely in a process known as “ultimate biodegradation”. The ergy- producing part of the metabolic activity consumes oxygen, resulting in the immediate formation of carbon dioxide, water and mineral salts in a process known as “mineralisation”.

Common pollutants

- Organic pollutants
- Petroleum
- Polymers
- Xenobiotics
- Pharmaceutics
- Tannery wastes
- Textile effluents
- Municipal wastes
- Agricultural effluents
- Fuels,

polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), pesticides and dyes are some of these types of compounds [9]. Some other synthetic chemicals like radionuclides and metals are extremely resistant to biodegradation by native flora compared with the naturally occurring organic compounds that are readily degraded upon introduction into the environment.

Examples

Aromatic compounds	Bacterial genera involved in degradation	References
Benzene, Toluene, Ethylbenzene, Xylene (BTEX)	*Pseudomonas*, *Rhodococcus*, *Mycobacterium*, *Burkholderia*, *Bacillus*, *Acinetobacter*	Jindrova et al. (2002), Wackett et al. (1988), Weelink, Eekert, and Stams (2010), Yavas & Icgen (2018), Zamanian and Mason (1987)
Halobenzenes (Chloro-/Fluoro-)	*Pseudomonas*, *Ralstonia*, *Burkholderia*	Reineke and Knackmuss (1988), van der Meer et al. (1991)
Nitrobenzenes	*Pseudomonas*, *Comamonas*	Nishino and Spain (1993, 1995)
Phthalate isomers and its esters	*Pseudomonas*, *Rhodococcus*, *Arthrobacter*, *Mycobacterium*, *Micrococcus*, *Gordonia*, *Comamonas*	Chatterjee and Dutta (2003), Stingley, Brezna, Khan, and Cerniglia (2004), Wang and Grady (1995), Sasoh et al. (2006)

Example Pesticide degradation

- Among the microbial communities, bacteria, fungi, and actinomycetes are the main transformers and pesticide degraders [49]. Fungi generally biotransform pesticides and other xenobiotics by introducing minor structural changes to the molecule, rendering it nontoxic. The biotransformed pesticide is released into the environment, where it is susceptible to further degradation by bacteria.
- Fungi and bacteria are considered as the extracellular enzyme-producing microorganisms for excellence.
- Enzyme-catalyzed degradation of a pesticide may be more effective than existing chemical methods.

Mechanism

- For pesticides degradation, three are mainly enzyme systems involved: **hydrolases, esterases (also hydrolases), the mixed function oxidases** (MFO), these systems in the first metabolism stage, and the glutathione S-transferases (GST) system,
- in the second phase [55]. Several enzymes catalyze metabolic reactions including hydrolysis, oxidation, addition of an oxygen to a double bound, oxidation of an amino group (NH_2) to a nitro group, addition of a hydroxyl group to a benzene ring, dehalogenation, reduction of a nitro group (NO_2) to an amino group, replacement of a sulfur with an oxygen, metabolism of side chains, ring cleavage.
- The process of biodegradation depends on the metabolic potential of microorganisms to detoxify or transform the pollutant molecule, which is dependent on both accessibility and bioavailability

- In Phase I metabolism, the initial properties of a parent compound are transformed through oxidation, reduction, or hydrolysis to generally produce a more water-soluble and usually a less toxic product than the parent.
- The second phase involves conjugation of a pesticide or pesticide metabolite to a sugar or amino acid, which increases the water solubility and reduces toxicity compared with the parent pesticide. The third phase involves conversion of Phase II metabolites into secondary conjugates, which are also non-toxic.
- In these processes fungi and bacteria are involved producing intracellular or extra cellular enzymes including hydrolytic enzymes, peroxidases, oxygenases, etc

Microbes

- Most notable among the pesticide degrading bacteria are **Pseudomonas, Bacillus, Flavobacterium, Alcaligenes, Arthrobacter.**