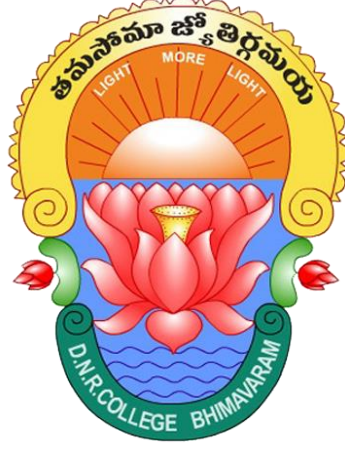


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BHIMAVARAM**

**DEPARTMENT OF PG MICROBIOLOGY
STUDY MATERIAL**



SEMESTER-IV

MBY-403: FOOD AND AGRICULTURE MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY

MBY- 403: FOOD AND AGRICULTURE MICROBIOLOGY

ROLE OF MICRO ORGANISMS:

- The role of micro organisms in the recycling of carbon bacteria and fungi contribute to the decay of the body.
- Micro organisms found on corpses early stages of decomposition:
- Bacillus , staphylococcus, candida streptococcus followed by:
- Salmonella , cytophaga , agrobacterium bacillus streptococcus salmonella.
- Micro organisms are collectively known as decomposers.
- Decomposers obtain a great source of energy from the body such as proteins, fats, organic carbohydrates and nucleic acids which are used as food source.
- This energy is then released through aerobic and anaerobic respiration.
- This energy allows rapid multiplication which leads to more decomposition.
- The role of micro organisms in the decomposition organic matter decomposition refers to the break down of the body which occurs following a death.
- Two components of decomposition decomposition is described as having two components
- Autolysis
- Putrefaction
- Autolysis- Autolysis refers to the situation where the body's own enzymes are acting on itself, causing cellular and tissue destruction.
- Putrefaction – putrefaction refers to the situation where micro organisms
- (especially bacteria and fungi) feed on and break down the tissues of the dead body.

Process of decomposition by micro organisms:

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- Within a very short time period micro organisms can break down and digest a large amount of soft tissue, resulting in a large production of gas and “decomposition fluid”.
- As decomposition proceeds, the skin begins to darken to various shades of green and brown.
- This is usually first seen within the right lower abdominal quadrant.
- The body becomes some what bloated due to the decomposition gases produced, and the decomposition fluids is frequently expelled from the mouth, nose or other opening in the presence of a red-brown fluid.
- Micro organisms are collectively known as decomposers. Other

decomposers:

- ❖ Insects are not the only organisms involved in decomposition of a body.
- ❖ Bacteria also plays a major role.
- ❖ Those found in the gut invade the dead tissues after death of a body as well as other fungi and bacteria from surroundings colonising the corpse.
- ❖ This in turn leads to decay.
- ❖ There is no set succession on the particular sequence of succession however genera often found on corpses include bacillus, candida, and argobacterium.
- ❖ These are collectively known as **decomposers**.

Micro organisms found on corpses:

- Early stages of decomposition:
- Bacillus
- Staphylococcus
- Candida
- Streptococcus

CARBON CYCLE

The carbon cycle is a fundamental process that regulates the Earth's carbon balance, impacting climate, ecosystems, and human activities. This essay explores the intricacies of the carbon cycle, its components, interactions, and significance in a comprehensive manner.

Introduction to the Carbon Cycle

The carbon cycle describes the movement of carbon atoms through various reservoirs on Earth: the atmosphere, oceans, land, and living organisms. It involves processes that transfer carbon between these reservoirs, maintaining a dynamic equilibrium crucial for life on our planet.

Carbon Reservoirs

1. **Atmosphere:** Carbon dioxide (CO₂) is a significant component (about 0.04% by volume) of the Earth's atmosphere. It is exchanged continuously with other reservoirs through processes like photosynthesis and respiration.
2. **Oceans:** The oceans store a massive amount of carbon in dissolved inorganic carbon (carbonate and bicarbonate ions) and organic matter. They absorb CO₂ from the atmosphere, influencing global climate and acidity levels.
3. **Terrestrial Biosphere:** Plants, soil, and detritus store carbon through photosynthesis, decomposition, and various geological processes. This reservoir plays a crucial role in the exchange of carbon with the atmosphere and oceans.

Processes in the Carbon Cycle

1. **Photosynthesis:** Plants and phytoplankton convert CO₂ and sunlight into organic carbon compounds (glucose) and oxygen through photosynthesis. This process removes CO₂ from the atmosphere.
2. **Respiration:** Organisms, including plants, animals, and microbes, release CO₂ back into the atmosphere by breaking down organic matter to obtain energy. This process is the opposite of photosynthesis.

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3. **Decomposition:** Dead organic matter is broken down by decomposers (bacteria and fungi), releasing CO₂ into the atmosphere or transforming it into organic carbon in the soil.
4. **Fossil Fuel Combustion:** Human activities, such as burning fossil fuels (coal, oil, natural gas), release vast amounts of CO₂ into the atmosphere, contributing significantly to global carbon emissions.
5. **Weathering and Erosion:** Geological processes, like weathering of rocks, dissolve CO₂ in water, which can lead to the formation of carbonate minerals and long-term carbon storage.

Global Carbon Balance

The carbon cycle maintains a delicate balance between carbon sources (emitters) and sinks (removers). Natural processes like photosynthesis, oceans, and geological storage act as sinks, absorbing more carbon than they emit. Human activities, primarily fossil fuel combustion and deforestation, disrupt this balance by releasing more carbon into the atmosphere than natural sinks can absorb, leading to an increase in atmospheric CO₂ levels and contributing to global warming.

Impact of Human Activities

1. **Climate Change:** Increased CO₂ levels in the atmosphere contribute to the greenhouse effect, trapping heat and leading to global warming and climate change.
2. **Ocean Acidification:** Higher CO₂ levels in the atmosphere lead to increased absorption by the oceans, causing ocean acidification. This has detrimental effects on marine life, especially organisms that rely on carbonate ions to build shells and skeletons.

Mitigation Strategies

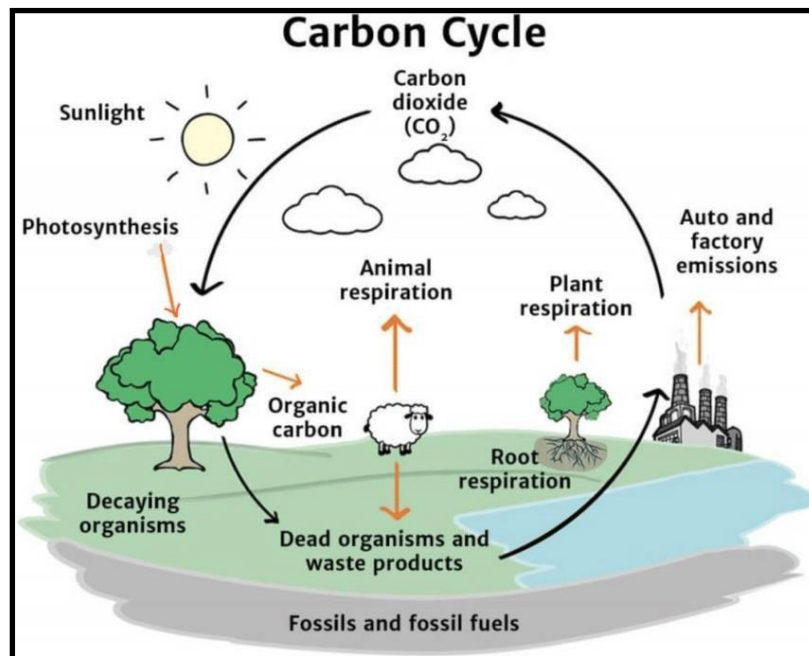
1. **Reducing Fossil Fuel Use:** Transitioning to renewable energy sources such as solar, wind, and hydroelectric power can reduce CO₂ emissions from energy production.
2. **Afforestation and Reforestation:** Planting trees and restoring forests can enhance carbon sequestration through photosynthesis, helping to offset emissions.

- Carbon Capture and Storage (CCS):** Technologies that capture CO₂ emissions from industrial processes and store them underground can mitigate emissions from sources that are challenging to eliminate completely.

Conclusion

The carbon cycle is a vital Earth system process that regulates climate, sustains ecosystems, and influences human activities profoundly. Understanding its components, interactions, and the impact of human activities is crucial for developing effective strategies to mitigate climate change and sustain a habitable planet for future generations. By addressing carbon emissions, enhancing natural carbon sinks, and promoting sustainable practices, we can work towards restoring the balance of the carbon cycle and minimizing the adverse effects of anthropogenic climate change.

In summary, the carbon cycle is a dynamic and intricate system that underscores the interconnectedness of Earth's processes and the urgency of global cooperation in addressing climate challenges.

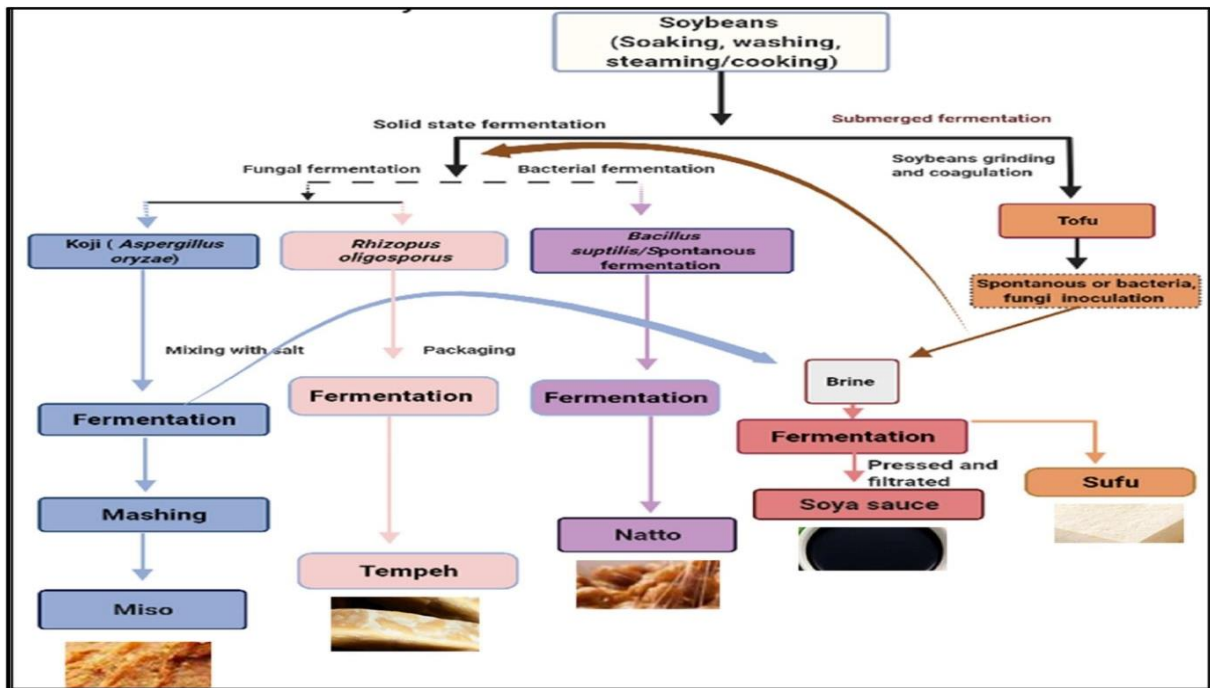


FERMENTED SOYA BEAN PRODUCTS:

- Fermented soybean products, including Soya sauce, Tempeh, Miso, and Natto have been consumed for decades, mainly in Asian countries.
- Beans are processed using either solid-state fermentation, submerged fermentation, or a sequential of both methods. Traditional ways are still used to conduct the fermentation processes, which, depending on the fermented products, might take a few days or even years to complete.
- Diverse microorganisms were detected during fermentation in various processes with *Bacillus* species or filamentous fungi being the two main dominant functional groups.
- Microbial activities were essential to increase the bean's digestibility, nutritional value, and sensory quality, as well as lower its antinutritive factors.
- The scientific understanding of fermentation microbial communities, their enzymes, and their metabolic activities, however, still requires further development.
- The use of a starter culture is crucial, to control the fermentation process and ensure product consistency.
- A broad understanding of the spontaneous fermentation ecology, biochemistry, and the current starter culture technology is essential to facilitate further improvement and meet the needs of the current extending and sustainable economy.
- This review covers what is currently known about these aspects and reveals the limited available information, along with the possible directions for future starter culture design in soybean fermentation.

Microbial ecology of traditional soybean fermentation

- Each country produces its own artisan soybean products, using different soybean fermentation procedures.
- Several factors influence the distinct characteristics of flavor and texture, including the type and length of fermentation processes, salt concentration, and moisture content, as well as additional components
- The manufacturing-specific details, different ingredients, and names used in different countries are out of this review scope and can be found elsewhere
- By understanding the microbial identities and their roles in different types of soybean fermentation, one can better control the fermentation processes and modulate the final product quality.
- Soybeans are generally subjected to either solid-state fermentation, submerged fermentation, or both.
- The microbial groups that lead the fermentation are mainly bacterial, fungal, or a mixture.
- the overall soybean process flow chart, the fermentation types, and the major microbial species that drive the processes.



Microbiology of soybean fermentation

- After preparation, soybeans are subjected to either solid-state, submerged fermentation processes, or a sequential of both processes.
- Solid-state fermentation is an old method of subjecting moist solid food particles to fermentation using bacteria, yeast, or filamentous fungi to produce fermented food or metabolites
 - such as enzymes, flavors, acids, etc.
 - Whole soybeans or their residues are subjected to bacterial or fungal solid-state fermentation to produce various fermented products including Natto, Tempeh, and the first stage of Miso, Soya sauce
- Each product has its characteristic flavor and texture because of the unique microbial populations found in the raw materials that dominate the fermentation.
- The submerged fermentation is a process conducted in the presence of free excess water. Two main soybean-fermented products are produced through a submerged fermentation stage, as part of the process in brine: Soya sauce and Sufu.
- **Natto**
 - Natto is produced by bacterial fermentation and characterized by a sweet, umami, and slightly bitter taste with the texture of a viscous polymer. Traditionally it was produced by wrapping boiled soybeans in rice straw pretreated with boiling water to kill vegetative microorganisms, leaving behind spore-forming *Bacillus* including *B. subtilis* subsp. *subtilis* (natto), and incubating it for about 3 days. Rice straw is also a natural habitat of other spore-forming *Bacillus* species that are believed to have a crucial impact on the fermented product, such as *B. amyloliquefaciens*, *B. pumilus*, *Bacillus licheniformis*, and *Bacillus atrophaeus* ;

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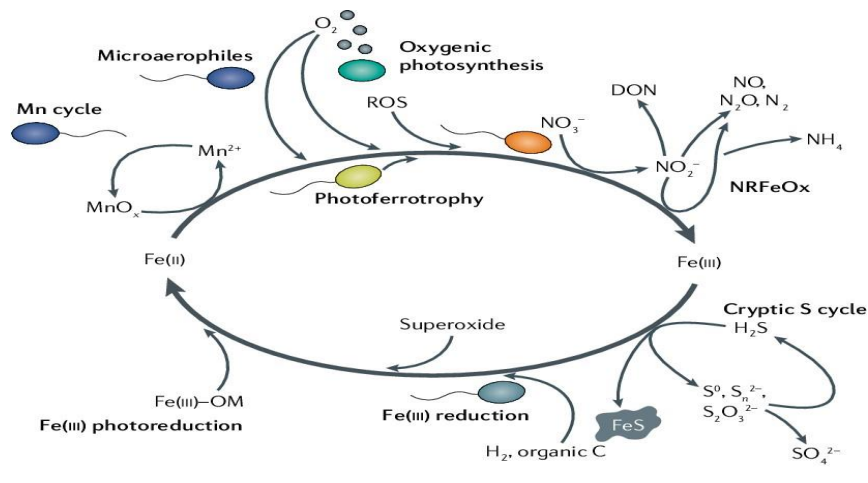
- Bacillus spores are known for their resistance to environmental stress, and their vegetative cells have high enzymatic activities to degrade protein, carbohydrates, and lipids During fermentation, fructan and polypeptides
- such as glutamic acid are produced which cover Natto with a stringy mucous material of unique flavor and aroma Furthermore, microbial lipolytic activities in soybeans cause the production of fatty acids such as oleic, linoleic, and linolenic
- acids with a significant impact on the final product's sensory quality The endogenous Bacillus species were reported to biosynthesize a wide range of alkyl pyrazines such as methylpyrazine, dimethyl
- They also produce inhibitory metabolites which suppress the growth of foodborne pathogens such as Escherichia coli, Bacillus cereus, Staphylococcus aureus, and Salmonella typhimurium and mycotoxin-producing fungi
- Kinema is a subtype of Natto that is also produced by spontaneous Bacillus fermentation.
- It has a sticky texture and ammoniacal odor
- B. subtilis was the predominant microbial isolate found during the Kinema fermentation and was believed to be attached to the bean surface.
- Besides, yeasts such as Candida parapsilosis and Geotrichum candidum, as well as bacteria, mainly Enterococcus faecium, were detected as originating from wooden mortar and pestle that were used to prepare soybeans
- The isolated Bacillus showed higher enzymatic activities including peptidase, phosphatase, lipase, and esterase compared with Enterococcus and the detected yeasts made the authors link the physicochemical changes that occurred in the fermented beans to Bacillus strains
- Premature arrest of alkaline fermentation (stuck fermentation) might be one of the challenging problems in soybean fermentation.
- The presence of organic acids, either naturally occurring during the soaking period from LAB fermentation or artificially introduced as previously described, may prevent this Bacillus fermentation To remove the organic acids and eliminate their negative impact on Natto production, flowing tap water is often applied after soaking.
- However, excessive flowing may affect the taste of Natto The mechanisms of such effects are largely unexplored and require further research, maybe by either optimizing the manufacturing process or selecting acid-tolerant Bacillus isolates to conduct the fermentation.

IRON CYCLE:

The **iron cycle** (Fe) is the biogeochemical cycle of iron through the atmosphere, hydrosphere, biosphere and lithosphere. While Fe is highly abundant in the Earth's crust, it is less common in oxygenated surface waters. Iron is a key micronutrient in primary productivity, and a limiting nutrient in the Southern ocean, eastern equatorial Pacific, and the subarctic Pacific referred to as High-Nutrient, Low- Chlorophyll (HNLC) regions of the ocean.

Iron exists in a range of oxidation states from -2 to +7; however, on Earth it is predominantly in its +2 or +3 redox state and is a primary redox-active metal on Earth. The cycling of iron between its +2 and +3 oxidation states is referred to as the iron cycle. This process can be entirely abiotic or facilitated by microorganisms, especially iron-oxidizing bacteria. The abiotic processes include the rusting of iron-bearing metals, where Fe^{2+} is abiotically oxidized to Fe^{3+} in the presence of oxygen, and the reduction of Fe^{3+} to Fe^{2+} by iron-sulfide minerals. The biological cycling of Fe^{2+} is done by iron oxidizing and reducing microbes.

Iron is an essential micronutrient for almost every life form. It is a key component of hemoglobin, important to nitrogen fixation as part of the Nitrogenase enzyme family, and as part of the iron-sulfur core of ferredoxin it facilitates electron transport in chloroplasts, eukaryotic mitochondria, and bacteria. Due to the high reactivity of Fe^{2+} with oxygen and low solubility of Fe^{3+} , iron is a limiting nutrient in most regions of the world.



A wide range of bacteria and archaea are capable of dissimilatory Fe^{3+} reduction. This includes genera from Euryarchaeota and Crenarchaeota, all five classes of

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Proteobacteria, Firmicutes, Deferribacteraceae, Acidobacteria, Thermotoga, and Thermus. This phylogenetic diversity may reflect the antiquity of FeH reduction. It is believed that early life began 3.5 billion to 3.8 billion years ago in an environment that was rich in Fe²⁺.

The photooxidation of Fe²⁺ to Fe³⁺ and H₂ would have provided an electron acceptor and energy source, respectively, to early cellular forms. Banded iron formation that occurred when atmospheric oxygen levels were beginning to increase at the end of the Precambrian era may be evidence of increased bacterial iron metabolism.

In addition to ferric iron (Fe³⁺) as a terminal electron acceptor, some magnetotactic bacteria such as *Aquaspirillum magnetotacticum* transform extracellular iron to the mixed valence iron oxide mineral magnetite (Fe₃O₄) and construct intracellular magnetic compasses.

Magnetotactic bacteria may be described as magnetoaerotactic bacteria because they are thought to use magnetic fields to migrate to the position in a bog or swamp where the oxygen level best meets their needs. Furthermore, some dissimilatory iron-reducing bacteria accumulate magnetite as an extracellular product.

Ferrous iron (Fe²⁺) can be used as an electron donor by lithotrophic microbes in acidic, oxic environments where Fe²⁺ is soluble and oxygen can serve as the terminal electron acceptor. This has been well characterized in the β-proteobacterium *Acidithiobacillus ferrooxidans* and thermophilic crenarchaeotes in the genus *Sulfolobus*.

In addition, it is now known that a number of microbes oxidize Fe²⁺ at neutral pH under oxic conditions. Best studied are γ-proteobacteria in the genus *Marinobacter* and β-proteobacteria in the genera *Leptothrix* and *Gallionella*.

Ferrous iron can also be oxidized under anoxic conditions with nitrate as the electron acceptor. One interesting anaerobic microbe is *Dechlorosoma suillum*, which oxidizes Fe²⁺ using perchlorate (ClO₄⁻) and chlorate (ClO₃⁻) as electron acceptors. Because perchlorate is a major component of explosives and rocket propellants, it is a frequent contaminant at retired munitions facilities and military bases.

- Iron is an important limiting nutrient for plants, which use it to produce chlorophyll. Photosynthesis depends on adequate iron supply. Plants assimilate iron from the soil into their roots.
- Animals consume plants and use the iron to produce hemoglobin, the oxygen transport protein found in red blood cells. When animals die, decomposing bacteria return iron to the soil.
- The marine iron cycle is very similar to the terrestrial iron cycle, except that phytoplankton and cyanobacteria assimilate iron.
- Iron fertilization has been studied as a method for sequestering carbon. Scientists have hoped that by adding iron to the ocean, plankton might be able to sequester the excess CO₂ responsible for climate change. However, there is concern about the long term effects of this strategy.

AZOLLA

INTRODUCTION

Azolla (mosquito fern, duckweed fern, fairy moss, water fern) is a genus of seven species of aquatic ferns in the family Salviniaceae. They are extremely reduced in form and specialized, looking nothing like other typical ferns but more resembling duckweed or some mosses. *Azolla filiculoides* is one of just two fern species for which a reference genome has been published. It is believed that this genus grew so prolifically during the Eocene (and thus absorbed such a large amount of carbon) that it triggered a global cooling event that has lasted to the present.

Azolla may establish as an invasive plant in areas where it is not native. In such a situation it can alter aquatic ecosystems and biodiversity substantially

Azolla (*Azolla pinnata*) is a heterosporous aquatic fern distributed all around the world. Azolla looks like other typical ferns that are green in colour however it looks more like duckweed or mosses, freely floating on the water surface. It can be used in animal and poultry feed as a protein source. Azolla has several pharmacological effects and can be used as antioxidant, immune-stimulating, hepato-protective, phytoremediation, bioremediation and also as nutritious material. Azolla contains vitamins (B12, beta carotene, vitamin A), biopolymers, minerals and amino acids. Azolla is rich in trace minerals and carotene. It looks to be potentially hepatoprotective drug against hepatotoxic substances. Its decoction (concentrated liquor of plant) has anti-inflammatory, antioxidant, and anti-apoptotic characteristics, making it an attractive preventive and therapeutic drug against super hepatotoxicity. Moreover, laying geese fed Azolla-based diets enhanced their FCR, overall performance, performance index, egg weight, egg production, egg shape index, and yolk colour. Thus, the goal of this explorative study was to learn more about the health advantages of Azolla, as well as to *Azolla pinnata*'s potential should be made known to scientists, veterinarians, and poultry nutritionists in order to boost chicken productivity.

REPRODUCTION

Azolla reproduces sexually, and asexually (by splitting). Like all ferns, sexual reproduction leads to spore formation, but unlike other members of this group Azolla is heterosporous, producing spores of two kinds. During the summer months, numerous spherical structures called sporocarps form on the undersides of the branches. The male sporocarp is greenish or reddish and looks like the egg mass of an insect or spider. It is two millimeters in diameter, and bears numerous male sporangia. Male spores (microspores) are extremely small and are produced inside each microsporangium. Microspores tend to adhere in clumps called massulae.

Female sporocarps are much smaller, containing one sporangium and one functional spore. Since an individual female spore is considerably larger than a male spore, it is termed a megaspore.

Azolla has microscopic male and female gametophytes that develop inside the male and female spores. The female gametophyte protrudes from the megaspore and bears a small number of archegonia, each containing a single egg. The microspore forms a male gametophyte with a single antheridium which produces eight swimming sperm. The barbed glochidia on the male spore clusters cause them to cling to the female megaspores, thus



facilitating fertilization.

USES

- Azolla has been extensively used as nitrogen fertilizer (Kumarasinghe & Eskew, 1993; Vlek et al., 1992) in especially paddy cultivation thus reducing cost of cultivation and increased paddy yield. Azolla is a great feed for livestock such as poultry, pigs, sheep, goat, fish, etc.
- Azolla (mosquito fern, duckweed fern, fairy moss, water fern) is a genus of seven

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species of aquatic ferns in the family Salviniaceae. They are extremely reduced in form and specialized, looking nothing like other typical ferns but more resembling duckweed or some mosses.

BENEFITS:

Compared to synthetic N-fertilizers, Azolla has various positive impacts on lowland rice production, including improving soil fertility, minimizing weeds, increasing soil organic carbon, improving microbial biomass, and thus nutrient cycling and enhancing rice growth and yield

APPLICATIONS.:

Azolla is an effective tool for enhancing the soil fertility, Bio- fuel production , Bio-remediation & Reducing GHGs emissions Enhancing crop productivity.

DIFFERENT TYPES OF AZOLLA:

- Each species has a specific native range: *Azolla caroliniana*, Eastern North America and the Caribbean
- *Azolla filiculoides*, Southern South America through Western North America including Alaska
- *Azolla microphylla*, tropical and subtropical America; *Azolla mexicana*, Northern South America through Western North America

CLASIFICATION:

- Azolla held by order Pteridophyta and the family Azollaceae. distributed in seven distinct species. It thrives in the tropics and subtropics, in different water bodies as stagnant water in sewers, rivers, ponds, canals and marshy areas
- Azolla is a small floating fern and is the only known pteridophyte that lives in symbiosis with a diazotrophic cyanobacterium. All the species of the genus harbour in their fronds a filamentous N₂-fixing cyanobacterium until now referred as *Anabaena azollae*.

PREPARATION OF YOGURT:

What is Yogurt?

- A yogurt is a fermented milk product that is made by combining two specific starter bacteria: *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.
- In yogurt, some natural derivatives of milk are added, for example, whey concentrates, skim milk powder, caseinates, or creams, to create a gel structure caused by the coagulation of milk proteins, as well as the presence of lactic acid secreted by defined species of bacteria, which must be present or abundant at the time of consumption.
- In addition to containing several essential nutrients, it also contains probiotics, fiber, vitamins, and minerals. Sweeteners, fruits, flavors, rice, soy, and nuts can also be added to it to change the consistency and aroma according to taste.
- In addition to its high digestibility and bioavailability, yogurt is considered a healthy food and can also be recommended to individuals with lactose intolerance, gastrointestinal disorders such as inflammatory bowel disease and irritable bowel disease, as well as helping with weight loss and immunity.

History of Yogurt:

- Around 5,000 years ago, yogurt was known to humans by nomadic people living in the Middle East.
- Throughout history, different civilizations have consumed it.
- It was named in the 8th century after the Turkish word “yogurtmak,” which means to thicken, coagulate, or curdle.
- Fermented milk products are mentioned in Indian Ayurvedic scripts dating back to about 6000 BC.
- In 1984, the FAO/WHO defined yogurt as “coagulated milk obtained by lactic acid fermentation by *Lactobacillus delbrueckii* spp. *Lactobacillus bulgaricus* (*Lb. bulgaricus*) and *Streptococcus thermophilus* from milk.
- At present, various names are used to refer to yogurts such as Dahi or Dahee in India, Roba in Iraq, Fiili in Finland, zabadi (Egypt), mast (Iran), lebenraib (Saudi Arabia), laban (Iraq and Lebanon), roba (Sudan), iogurte (Brazil), cuajada (Spain), coalhada (Portugal), dovga (Azerbaijan), and matsoni (Georgia, Russia, and Japan).

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- Today, many forms of yogurt can be found, including plain yogurt, fruit-flavored yogurt (including fruit-on-the-bottom and blended forms), whipped yogurt, granola-topped yogurt, drinkable yogurt, frozen yogurt, and Greek yogurt with varying fat contents (regular, low fat, and non-fat).

Ingredients of Yogurt:

- Yogurt is a dairy product containing a variety of ingredients.
- The main ingredient is milk; sweeteners, stabilizers, fruits, flavors, and bacteria are also added.
- To make yogurt, the type of milk used depends on the desired fat content.
- Whole milk is used for full-fat or regular yogurt. Skim milk is used for fat-free yogurt, while partially skimmed milk is preferred for low-fat yogurt.
- The fat content of the yogurt mixture can be adjusted by adding cream or butterfat, while the solid content can be improved with skim milk powder and whey protein concentrate.
- Adding stabilizers to the mixture gives yogurt a smooth texture, whey separation is prevented, and ingredients are distributed evenly. Sweeteners are added to yogurt to enhance its flavor and aroma.

Manufacture of Yogurt:

Yogurt is made through several steps, including blending, pasteurization, inoculation and fermentation, and cooling.

1. Pasteurization:

- It is the first step in ensuring the product's safety.
- During pasteurization, all pathogenic bacteria in the milk are killed, reducing the number of other organisms significantly and inactivating the enzymes in the milk.
- Generally, yogurt is pasteurized using a high-temperature short-time heat with a long holding time.
- Whey proteins are denatured by heat treatment and optimal conditions are created for yogurt culture growth.
- Denaturing whey proteins (80%–85%) improves their water binding capacity, which reduces free whey separation and improves yogurt consistency and viscosity.

2. Inoculation:

- The yogurt mixture is cooled to 43-46°C after pasteurization.

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- Then 2-3% of yogurt starter culture bacteria are added to the mixture, usually *Staphylococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in a 1:1 ratio.
- A constant incubation temperature is maintained throughout the manufacturing process for yogurt.
- As the pH of yogurt approaches 5.0, microorganisms begin to operate, gradually dominating the fermentation process until the desired pH and acidity are reached.
- At 4°C, the process terminates, but the culture remains alive but has a limited ability to grow.
- In the fermentation process, lactose is converted into lactic acid by the lactic acid bacteria, which aids in the coagulation of milk proteins and the production of volatile compounds.

3. Cooling:

- The final step is cooling, which consists of two phases.
- As the coagulum temperature drops rapidly below 10°C, fermentation is inhibited and yogurt with low viscosity is produced.
- In the second phase of cooling, the coagulum temperature decreases rapidly to less than 20°C, and then the temperature of yogurt is lowered down to 5°C (storage temperature) with increased viscosity.
- Upon manufacturing, set yogurts are directly transferred to cold storage for chilling, whereas stirred yogurts are first cooled down by agitation before being filled.

Microbiology of Yogurt:

- The production of yogurt needs to begin with selecting high-quality milk that meets the necessary microbiological and chemical standards.
- Usually, milk is heated to a high temperature, between 85°C and 90°C, for a short time to sterilize it and denature the whey proteins.
- The purpose of this step is to ensure that any unwanted microorganisms are destroyed, which could spoil the yogurt.
- The milk is cooled to 43°C to 46°C after sterilization, which is an ideal growth temperature for *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, which are commonly used in yogurt production.
- Lactose is fermented by the bacteria when added to milk as a mixed culture or separately.
- When bacteria consume lactose, they produce lactic acid, which drops the pH of milk and produces a solid gel-like texture by coagulating milk proteins.

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- In yogurt, acidification is the key microbiological process responsible for giving it its characteristic thick and tangy texture.
- The fermentation process typically lasts four to eight hours, during which bacteria continue to consume lactose and produce lactic acid, further acidifying and thickening it.
- To ensure the fermentation process proceeds optimally and the final product meets the desired acidity and texture, the temperature and pH of the mixture are carefully controlled.
- Upon reaching the desired level of acidification and texture, the yogurt is cooled to stop fermentation and stabilize it.
- During this step, fruit, honey, or granola can be added as flavorings, sweeteners, or other ingredients.
- Yogurt is then packaged and distributed for consumption.

Types of Yogurt:

Based on the physical nature

1. Set yogurt
2. Stirred yogurt

Based on Flavor:

1. plain/natural yogurt
2. Flavored yogurt

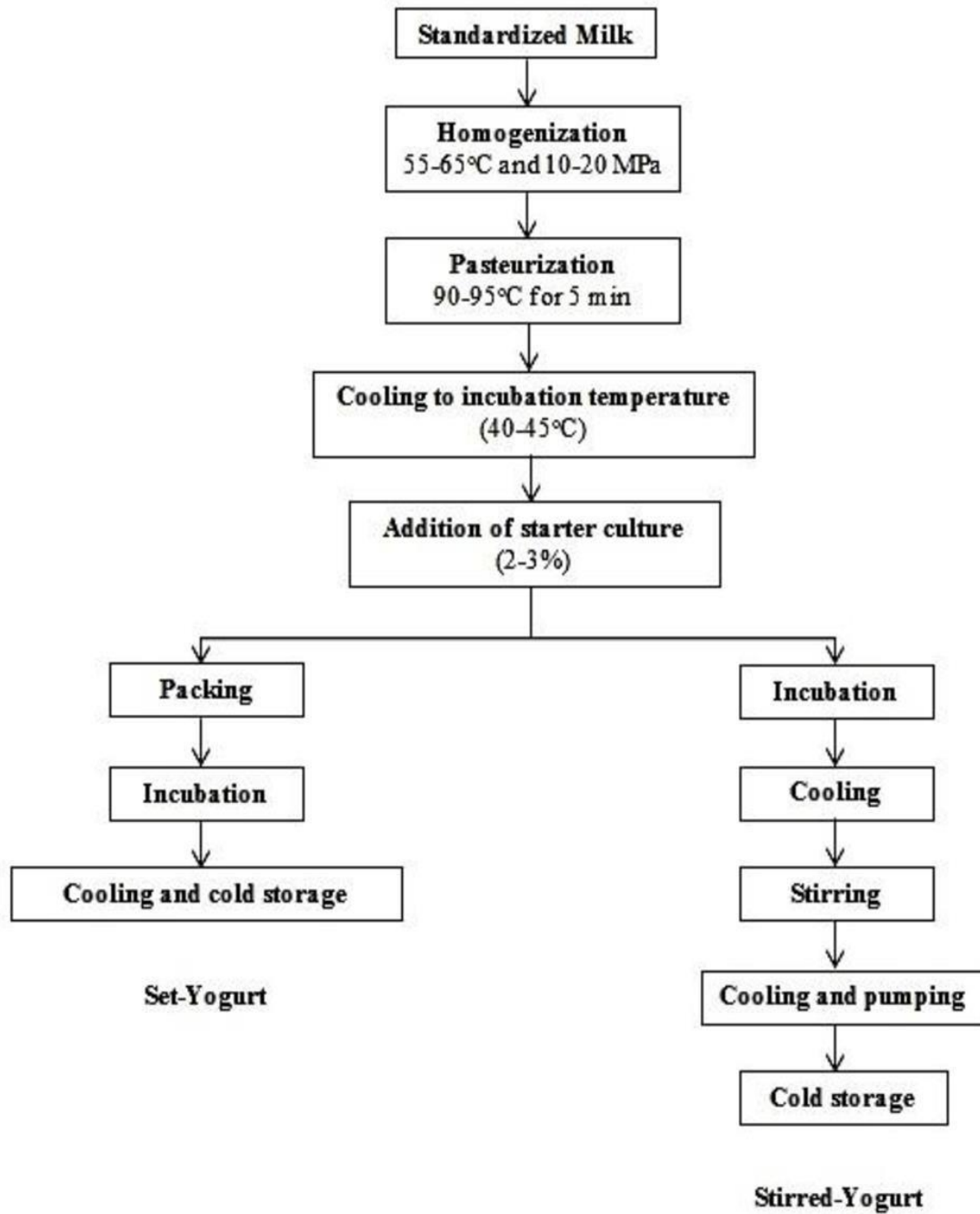
Based on the production method:

1. Pasteurized and UHT yogurt
2. Frozen yogurt
3. Dried yogurt/yogurt powder
4. Herbal yogurt

Health Benefits of Yogurt:

- Yogurt containing active cultures may benefit gut health.
- Yogurt contains certain probiotic strains that may boost the immune system.
- High blood pressure and osteoporosis may be prevented by yogurt.
- Yogurt can help you recover post-workout as it is a high-protein food.
- The probiotics found in yogurt can improve digestion, metabolic health, and immunity, as well as delay or prevent cancer's onset.
- The consumption of yogurt can result in fat burning and may protect against colon cancer.
- Yogurt may also lower the risk of vaginal infections and strengthen collagen in the skin.

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MYCORRHIZA:

Definition: A mycorrhiza is a symbiotic association between a green plant and a fungus. The plant makes organic molecules by photosynthesis and supplies them to the fungus in the form of sugars or lipids, while the fungus supplies the plant with water and mineral nutrients, such as phosphorus, taken from the soil.

Types of Mycorrhiza:

The two main types of mycorrhizae are ectomycorrhizae and endomycorrhizae. They are categorised according to where the fungi colonise on the plants.

Ectomycorrhiza:

Ectomycorrhiza, also known as EcM, usually develops connections between woody plants (like beech, birch, willow, oak, pine, fir and spruce) and fungi belonging to the Ascomycota, Basidiomycota, and Zygomycota. About 10% of plant families have ectomycorrhizal relationships.

Ectomycorrhizas comprise a Hartig net of hyphae encircling the plant cells in the root cortex and a mantle or hyphal sheath covering the root tip. The term “ectomycorrhiza” refers to a mycorrhiza in which the hyphae have the potential to enter the plant cells.

Springtails are attracted to and killed by the ectomycorrhizal fungus *Laccaria bicolor* to extract nitrogen, some of which may be passed to the mycorrhizal host plant.

Endomycorrhiza:

Alternatively, endomycorrhizae are present in more than 80% of plant families, including greenhouse and crop plants such as vegetables, flowers, grasses, and fruit trees. The production of vesicles and arbuscules by the fungus and their penetration of the cortical cells are characteristics of endomycorrhizal relationships.

There are several types of endomycorrhizas including arbuscular, arbutoid, ericoid, monotropoid and orchid mycorrhizas.

Arbuscular Mycorrhiza:

In an arbuscular mycorrhiza, also known as an AMF, the symbiotic fungus reaches the cortical cells of the roots of a vascular plant to produce arbuscules. Only members of the division Glomeromycota of fungi can produce arbuscular mycorrhizas.

Ericoid Mycorrhiza:

Ericaceae plants and various mycorrhizal fungal lineages come together to produce the mutualistic association known as the ericoid mycorrhiza. It has also been demonstrated that ericoid mycorrhizas are highly saprotrophic, allowing plants to obtain nutrients from still-in-decomposition materials through the decomposing activities of their ericoid companions.

Monotropoid Mycorrhiza:

This kind of mycorrhiza can be found in various genera of the Orchidaceae and the Ericaceae subfamily Monotropoideae. These plants get their carbon from the fungal companion and are heterotrophic or mixotrophic. Thus, this type of mycorrhizal relationship is parasitic and non-mutualistic.

Orchid Mycorrhiza:

Every orchid undergoes myco-heterotrophic growth at some point in its life cycle, forming orchid mycorrhizas with various basidiomycete fungi. Their hyphae enter the root cells and create pelotons (coils) to exchange nutrients.

Mycorrhiza biofertilizer production

Mycorrhizal fungi are often used in fertilizer production due to their ability to enhance nutrient uptake in plants. They form symbiotic relationships with plant roots, improving nutrient absorption and overall plant health. Fertilizer formulations incorporating mycorrhiza aim to boost crop yields and reduce the need for chemical inputs, promoting sustainable agriculture practices.

Steps:

1. Isolation and Selection of Mycorrhizal Fungi
2. Cultivation and Propagation
3. Formulation Development
4. Quality Control and Testing
5. Packaging and Storage
6. Distribution and Marketing
7. Application

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1. Isolation and Selection of Mycorrhizal Fungi:

- Identify and isolate specific strains of mycorrhizal fungi known to form beneficial symbiotic relationships with plants.
- Select fungi based on their compatibility with target plant species and their ability to thrive in specific soil conditions.

2. Cultivation and Propagation:

- Grow the isolated mycorrhizal fungi in laboratory or greenhouse conditions.
- Provide suitable growth media that support fungal development and reproduction.
- Monitor growth parameters such as temperature, pH, and nutrient levels to optimize fungal biomass production.

3. Formulation Development:

- Mix the cultivated mycorrhizal fungi with carrier materials that provide a stable environment for the fungi and facilitate even distribution during application.
- Select carrier materials such as vermiculite, peat, or clay that are inert and do not interfere with fungal viability.
- Incorporate additives or nutrients that enhance fungal growth or improve plant nutrition, depending on the intended application.

4. Quality Control and Testing:

- Conduct rigorous testing to assess the viability and purity of the mycorrhizal inoculant.
- Perform quality assurance checks to ensure the product meets specified standards for fungal concentration and effectiveness.
- Test the inoculant in greenhouse or field trials to validate its performance in enhancing plant growth and nutrient uptake.

5. Packaging and Storage:

- Package the mycorrhizal inoculant in appropriate containers that maintain fungal viability and shelf life.
- Ensure packaging materials protect the inoculant from environmental factors such as moisture and sunlight that could degrade fungal spores.

6. Distribution and Marketing:

- Distribute the mycorrhizal fertilizers to agricultural suppliers, nurseries, or directly to consumers.
- Provide educational materials and technical support to inform users about the benefits of using mycorrhizal inoculants and proper application techniques.

7. Application :

- Instruct end users on how to apply the mycorrhizal inoculant to soil or directly to plant roots.
- Encourage regular application to establish and maintain beneficial fungal populations in the root zone, enhancing plant health and productivity.

By following these comprehensive steps, producers can ensure they develop high-quality mycorrhizal fertilizers that effectively support sustainable agricultural practices and improve crop yields.

Methods of Inoculum Production and Inoculation:

Methods of inoculum production of VAM fungi differ; however, some of these two are briefly described here.

(a) Ectomycorrhizal fungi:

The basidiospores, chopped sporocarp, sclerotia, pure mycelia culture, fragmented mycorrhizal roots or soil from mycorrhizosphere region can be used as inoculum. The inoculum is mixed with nursery soils and seeds are sown.

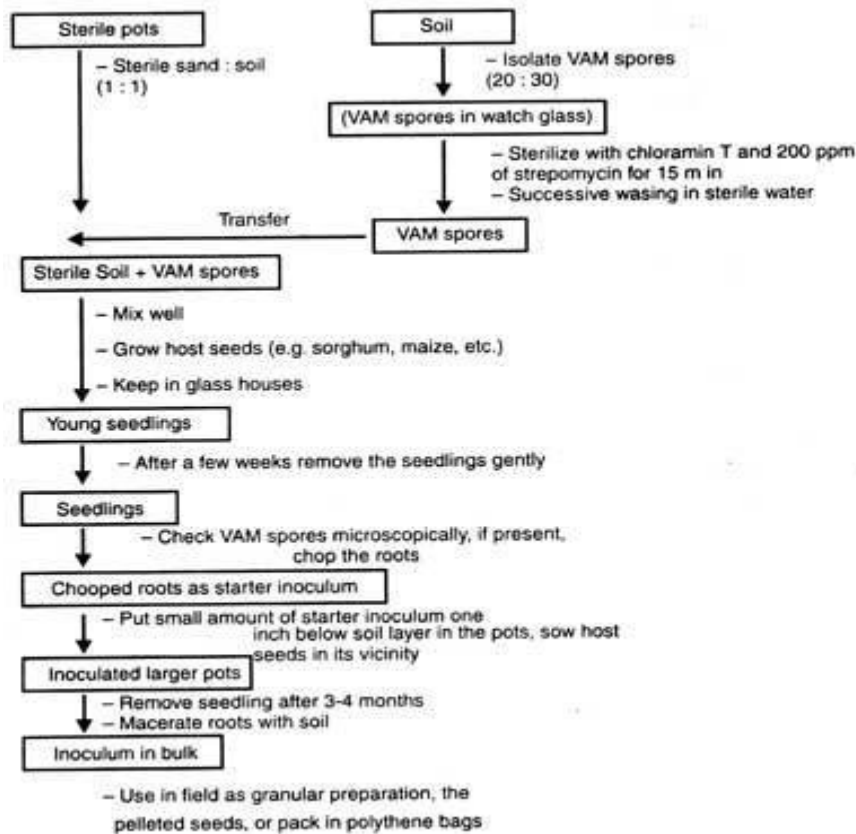
(b) VA mycorrhizal fungi :

VA mycorrhizas can be produced on a large scale by pot culture technique. This requires the host plants, mycorrhizal fungi and natural soil. The host plants which support large scale production of inoculum are sudan grass, strawberry, sorghum, maize, onion, citrus, etc.

The starter inoculum (spores) of VA mycorrhizal fungi can be isolated from soil by wet sieving and decantation technique (Gerdeman and Nicolson, 1963). VA mycorrhizal spores are surface sterilized and brought to the pot culture. Commonly used pot substrates are sand ,soil with a

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little amount of moisture. There are two methods of using the inoculum : (i) using a dried spore-root- soil to plants by placing the inoculum several centimeters below the seeds or seedlings, (ii) using a mixture of soil-roots, and spores in soil pellets and spores adhered to seeds with adhesives.



Uses:

- **Boosting Nutrient Absorption:** Helps plants take up more nutrients like phosphorus and nitrogen from the soil.
- **Promoting Plant Growth:** Enhances overall growth, including root development and crop yield.
- **Improving Drought Resistance:** Helps plants survive better during dry periods by improving water uptake.
- **Protecting Plants:** Can make plants more resistant to certain diseases.

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- **Environmentally Friendly:** Supports healthy soil and reduces the need for chemical fertilizers.
- **Used in Organic Farming:** Compatible with organic farming methods.
- **Cost-Effective:** Can lead to higher crop yields and reduce costs for farmers.