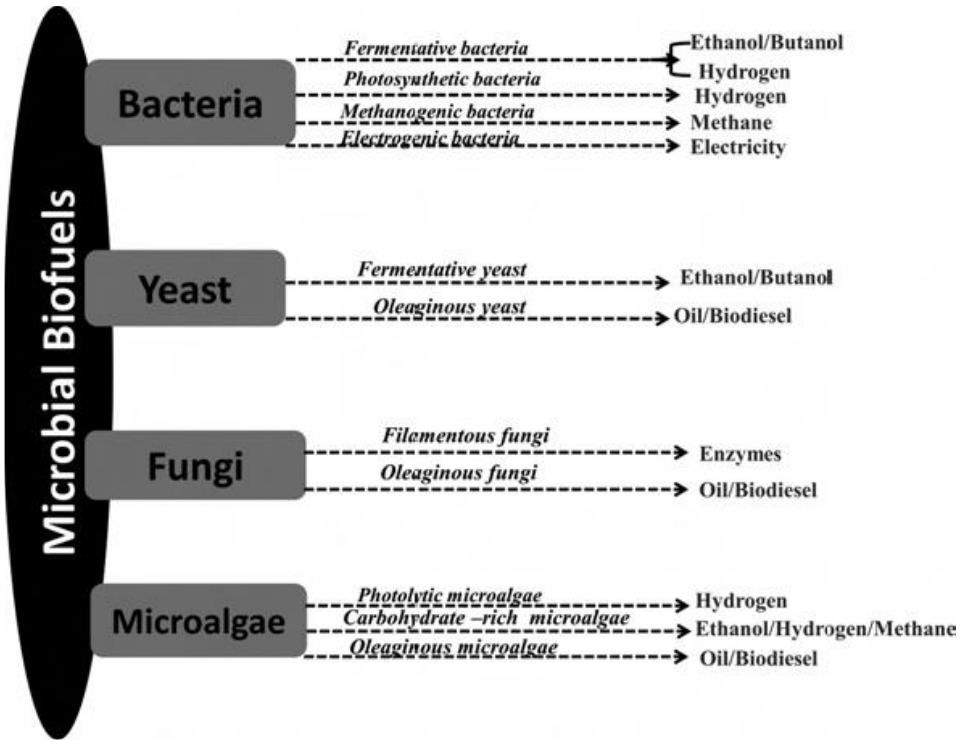
Microorganisms Involved in Biofuel Production Processes

The biochemical processes have an advantage over thermochemical processes because they are less energy intensive and are environmentally friendly.

Microorganisms play a crucial role in the biochemical conversion of biomass. Different group of microbes such as bacteria, yeasts, and fungi have innate capabilities to generate different value-added products through their metabolic mechanisms.



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Selection of suitable microbes for biofuel production

Biomass feedstocks—from simple sugars to complex organic matter—can be converted to biofuels, and microbial fermentation is the most efficient method with low energy input. However, biofuel yield depends heavily on the microorganism used.

An ideal microbial host should have:

1. high biodegradability of various carbohydrates,
2. high product yield,
3. tolerance to end-product toxicity,
4. resistance to environmental stress,
5. flexibility in substrates and products,
6. genetic competence for improvement.

Because no single strain possesses all these traits, screening is necessary to identify the most suitable microbes for biofuel production.

❖ Bacterial biofuels

Bacteria are the most widely studied microorganisms for biofuel production due to their abundance, rapid growth, ability to use diverse substrates, and capability to produce various metabolic products. Their metabolic pathways can be easily modified to obtain specific biofuels. According to their biofuel-producing mechanisms, bacteria are generally classified into photosynthetic, fermentative, methanogenic, and electrogenic groups.

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Different Genera of Bacteria Used for Biofuel Production

Organism	Type	Mechanism	Biofuel
Synechocystis PCC 6803	Cyanobacteria	Direct biophotolysis	Hydrogen
Plectonema boryanum	Cyanobacteria	Indirect biophotolysis	Hydrogen
Anabaena variabilis	Cyanobacteria	Indirect biophotolysis	Hydrogen
Chloroflexus aurantiacus	Green-gliding bacteria	Photofermentation	Hydrogen
Rhodobacter sphaeroides	Purple non-sulfur bacteria	Photofermentation	Hydrogen
Thiocapsa roseopersicina	Purple non-sulfur bacteria	Photofermentation	Hydrogen
Zymomonas mobilis	Fermentative bacteria	Ethanol fermentation	Ethanol
Thermoanaerobacter ethanolicus	Fermentative bacteria	Ethanol fermentation	Ethanol
Clostridium thermohydrosulfuricum	Fermentative bacteria	Ethanol fermentation	Ethanol
Butyribacterium methylotrophicum	Fermentative bacteria	Mixed acid fermentation	Ethanol
Clostridium acetobutylicum	Fermentative bacteria	Acetone-Butanol-Ethanol fermentation	Butanol
Clostridium beijerinckii BA101	Fermentative bacteria	Acetone-Butanol-Ethanol fermentation	Butanol
Clostridium toanum	Fermentative bacteria	Mixed acid fermentation	Butanol
Clostridium butyricum strain 4P1	Fermentative bacteria	Mixed acid fermentation	Methanol
Clostridium propionicum	Fermentative bacteria	Mixed acid fermentation	n-Propanol
Clostridium toanum	Fermentative bacteria	Acetone-Butanol-Isopropanol fermentation	Isopropanol

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1. Photosynthetic Bacteria

Photosynthetic bacteria generate hydrogen using sunlight, water, and various substrates. They contain pigments and reaction centers similar to plants, enabling them to convert light energy into chemical energy. Hydrogen production in these bacteria occurs through two main mechanisms: ***biophotolysis*** and ***photofermentation***.

1) Biophotolysis

Biophotolysis is the process in which water is split into molecular hydrogen and oxygen in Direct Biophotolysis or Indirect Biophotolysis by the action of sunlight as in the following:

$$2H2O + hv\,(light\ energy) \rightarrow 2H2 + O2$$

2) Photofermentation

Unlike cyanobacteria, purple and green photosynthetic bacteria are anoxygenic, meaning they do not produce oxygen. These bacteria can generate hydrogen through photofermentation, using many different inorganic and organic substrates in the presence of light.

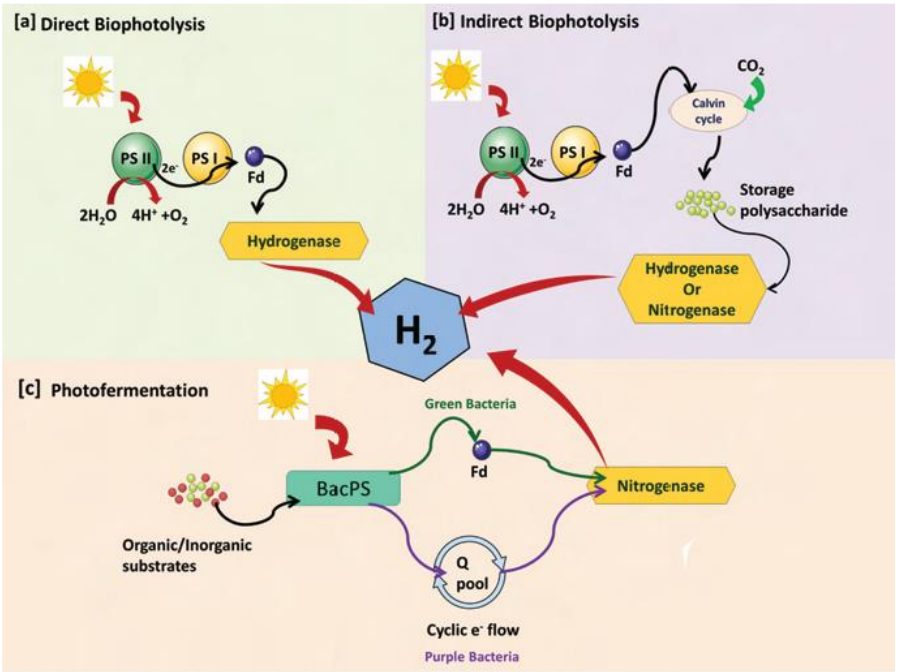

Figure 1

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2. Heterotrophic Fermentative Bacteria

The wide range of products formed through bacterial fermentation pathways makes fermentative microorganisms valuable for biofuel production. Their simple metabolic systems allow targeting specific products and improving yields through process engineering or genetic modification. The same bacteria can also produce different valuable metabolites under different growth conditions. Various fermentation pathways—often named after their major end products—include ethanol fermentation, acetone–butanol fermentation, homoacetic acid fermentation, and mixed acid fermentation.

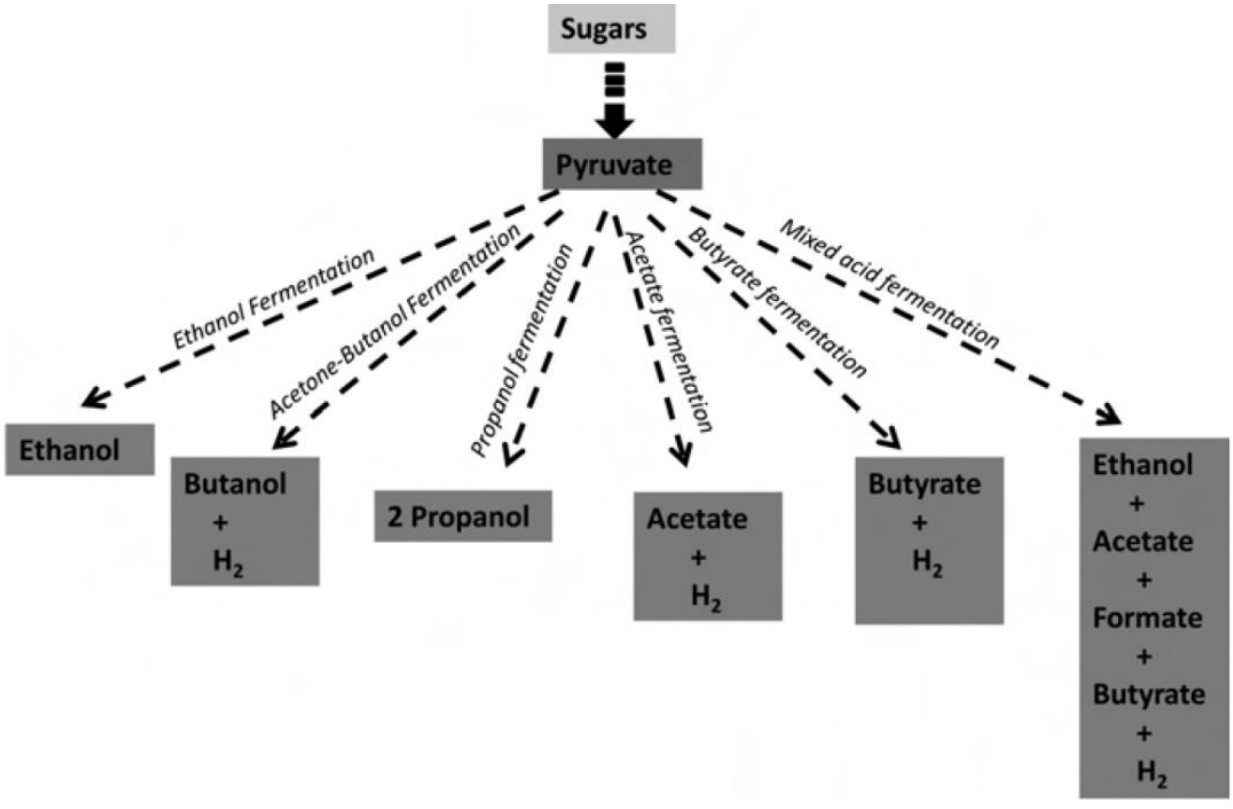

Figure 2 Different fermentative pathways for biofuel production.

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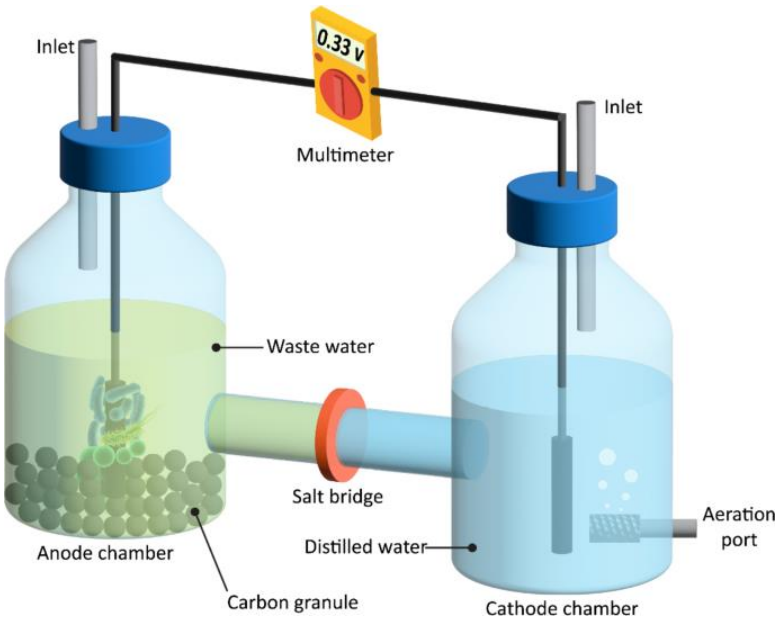
3. Methanogenic Bacteria

Biomethane is traditionally produced by anaerobic digestion and has long been used as a household fuel. In this process, methanogens convert organic acids—formed during the breakdown and fermentation of organic waste—into methane. These microorganisms typically live in extreme conditions and prefer a pH of 6–9. Methanogens are strict anaerobes and are generally classified into two groups based on their substrates: acetoclastic (using acetate) and hydrogenotrophic (using hydrogen and CO_2).

4. Electrogenic Bacteria

These organisms often are used in different applications such a microbial fuel cells and microbial electrolysis to harness electricity and fuel (hydrogen), respectively. Such electron-releasing bacteria, called exoelectrogens or electrogens, transfer electrons from inside the cell to external metal surfaces. Depending on the species, this transfer may occur through mediated electron transfer (using external or internal electron shuttles) or direct transfer.



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❖ Yeast biofuel

Yeast, mostly Saccharomyces cerevisiae, has been traditionally used for commercial bioethanol production for several decades. It is used because of its characteristic features such as

1) low cost,
2) high ethanol productivity,
3) easy availability,
4) high tolerance, and
5) flexibility of substrates.

Disadvantage:

The major challenge of yeast fermentation is that it cannot convert pentoses to ethanol and thus lowers the substrate conversion efficiency when using organic wastes as substrates.

Besides bioethanol, yeasts also have been explored for use in alternate advanced biofuel production. The yeast cell factory, Saccharomyces cerevisiae, has been metabolically engineered to produce other higher alcohols like butanol and isobutanol.

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❖ Biofuel from filamentous fungi

Although yeasts are also a subclass of fungi, the characteristic difference between them and the filamentous fungi has brought a massive distinction among these organisms. Like yeasts certain filamentous fungi exhibit the capacity to accumulate intracellular lipids during metabolic stress periods. Most of the yeasts contain lipids in the form of triacylglycerols (TAG). By altering the growth conditions, the accumulation of lipids in the yeasts can be enhanced significantly. The different filamentous fungal species that have been reported for biodiesel production include Cunninghamella echinulata, Mortierella isabellina, Mucor circinelloides, Mortierella alpine, Aspergillus niger, and Rhizopus oryzae. Filamentous fungi can accumulate up to 80% weight per weight (%w/w) lipids. However, not all these lipids are useful for biodiesel production. It was suggested that only saponifiable lipids and free fatty acids can be converted to fatty acid methyl esters.

Using filamentous fungi for biodiesel production has several **advantages**;

1) it has high growth rates and thus can rapidly accumulate storage lipids,
2) it shows good lipid profiles and thus can be converted to high quality biodiesel,
3) it can use a variety of carbon sources, and
4) due to its tendency to form cell pellets, the harvesting process can be simplified.

Disadvantages:

1) the cost of the production process which needs to be addressed for the commercial viability of the process.
2) Furthermore, the strains available have only 20%–25% usable lipids for biodiesel production

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❖ Microalgal biofuel

Microalgae are microscopic algae that are found in freshwater and marine water ecosystems. They have higher growth rates and can be grown in non-arable lands. Certain microalgae have the capability to alter their chemical composition according to the change in the environmental conditions. ***This property has marked potential for use of these organisms in biofuel production***. Microalgae can be converted to bioethanol, biohydrogen, biomethane, and biodiesel using different biochemical and thermochemical processes. Microalgae has starch as its storage carbohydrate, which can be used as a feedstock for the bacterial or the yeast fermentation process for bioalcohols or biogas production.

Microalgae have an **advantage** over fungi because

1) they can be grown photosynthetically.
2) Furthermore,the ability of microalgae to fix atmospheric CO_2 can aid in lowering the greenhouse gas emissions, which is environmentally sustainable.

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Q1/ Multiple-Choice Questions (MCQs)

1. Which advantage do biochemical processes have over thermochemical processes?
   a) Higher energy consumption
   b) More pollution
   c) Less energy intensive and environmentally friendly
   d) Require extreme temperatures
   Answer: c

2. Which group of microorganisms is most widely studied for biofuel production?
   a) Viruses
   b) Bacteria
   c) Protozoa
   d) Algae
   Answer: b

3. Which of the following is NOT a trait of an ideal microbial host?
   a) High product yield
   b) Tolerance to end-product toxicity
   c) Zero genetic flexibility
   d) Resistance to environmental stress
   Answer: c

4. Hydrogen production in photosynthetic bacteria occurs mainly through:
   a) Hydrolysis
   b) Biophotolysis and photofermentation
   c) Electrolysis
   d) Methanogenesis
   Answer: b

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5. Which type of bacteria converts organic acids into methane?
   a) Electrogenic bacteria
   b) Methanogenic bacteria
   c) Fermentative bacteria
   d) Photosynthetic bacteria
   Answer: b

6. The major disadvantage of yeast fermentation is its inability to:
   a) Grow rapidly
   b) Produce ethanol
   c) Convert pentoses to ethanol
   d) Tolerate high ethanol levels
   Answer: c

7. Filamentous fungi are useful for biodiesel production mainly because they can accumulate:
   a) Water
   b) Proteins
   c) Intracellular lipids
   d) Carbohydrates
   Answer: c

8. Microalgae have an advantage over fungi because:
   a) They cannot photosynthesize
   b) They grow only in soil
   c) They grow photosynthetically and fix CO_2
   d) They produce only methane
   Answer: c

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Q2/ True / False

1. Microorganisms can convert a wide range of biomass feedstocks into biofuels. **True**
2. All microorganisms possess all ideal traits required for efficient biofuel production. **False**
3. Photofermentation by purple and green bacteria produces oxygen. **False**
4. Fermentative bacteria can produce different metabolites depending on growth conditions. **True**
5. Methanogens are strict anaerobes and prefer a pH range of 6–9. **True**
6. Yeast has high ethanol productivity and good tolerance. **True**
7. All lipids from filamentous fungi can be converted to biodiesel. **False**
8. Microalgae can be used to produce bioethanol, biohydrogen, biomethane, and biodiesel. **True**

Q3/ Fill in the Blanks

1. Biochemical processes are less _____ intensive and more environmentally friendly than thermochemical processes. **Answer: energy**
2. Microbial fermentation is the most efficient method for converting biomass because it requires low _____. **Answer: energy input**
3. Hydrogen production in photosynthetic bacteria occurs through _____ and _____. **Answer: biophotolysis, photofermentation**
4. Fermentation pathways are often named after their major _____. **Answer: end products**
5. Methanogens are classified into two types: _____ and _____. **Answer: acetoclastic, hydrogenotrophic**
6. The most common yeast used in commercial ethanol production is _____. **Answer: Saccharomyces cerevisiae**
7. Filamentous fungi can accumulate up to _____% w/w lipids. **Answer: 80**
8. Microalgae store carbohydrates mainly in the form of _____. **Answer: starch**

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Q4/Short-Answer Questions

1. **Why does biofuel yield depend heavily on the microorganism used?**
   Answer: Because microorganisms differ in their
   1) biodegradability of substrates,
   2) product yield,
   3) tolerance to toxicity,
   4) substrate flexibility,
   5) environmental resistance,
   6) genetic competence.

2. **What is the main difference between biophotolysis and photofermentation?**
   Answer: Biophotolysis splits water to produce hydrogen and oxygen, while photofermentation uses organic and inorganic substrates without producing oxygen (anoxygenic)

3. **What conditions do methanogenic bacteria prefer?**
   Answer: Strict anaerobic environments with a pH between 6 and 9.

4. **What is a major limitation of using yeast for bioethanol production?**
   Answer: Yeast cannot convert pentose sugars to ethanol, reducing conversion efficiency when using complex waste substrates.

5. **Why are filamentous fungi considered good candidates for biodiesel production?**
   Answer: They grow quickly, accumulate high levels of lipids, use diverse carbon sources, and form pellets that simplify harvesting.

6. **How can microalgae contribute to environmental sustainability?**
   Answer: By fixing atmospheric CO_2 during photosynthesis, reducing greenhouse gas emissions.

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7. **Which biofuels can microalgae produce?**
   Answer: Bioethanol, biohydrogen, biomethane, and biodiesel.

8. **List the advantages of using filamentous fungi for biodiesel production.**
   Answer:
   1) high growth rates and rapid lipid accumulation
   2) good lipid profiles suitable for high-quality biodiesel
   3) ability to use a variety of carbon sources
   4) tendency to form cell pellets, simplifying harvesting

9. **List the disadvantages of using filamentous fungi for biodiesel production.**
   Answer: high production cost **and** only 20%–25% of lipids are usable for biodiesel

10. **What are the advantages of biochemical processes compared to thermochemical processes?**
    Answer: less energy intensive **and** environmentally friendly

11. **What are the characteristic features (advantages) of yeast used in bioethanol production?**
    Answer:
    1) low cost
    2) high ethanol productivity
    3) easy availability
    4) high tolerance
    5) flexibility of substrates

12. **What is the main disadvantage of yeast fermentation?**
    Answer: cannot convert pentoses to ethanol **and** lowers substrate conversion efficiency when using organic wastes

13. **What makes filamentous fungi different and useful in lipid accumulation for biodiesel?**

Answer:
capacity to accumulate intracellular lipids during metabolic stress and accumulation enhanced by altering growth conditions

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14. Give examples of the two main types of methanogens.
Answer: acetoclastic methanogens **and** hydrogenotrophic methanogens

15. Give examples of fermentation pathways used by fermentative bacteria.
Answer:

1) ethanol fermentation
2) acetone–butanol fermentation
3) homoacetic acid fermentation
4) mixed acid fermentation

16. List the two mechanisms used by photosynthetic bacteria to produce hydrogen.
Answer: biophotolysis and photofermentation

17. List the types of biofuels produced from microalgae.
Answer:

1) bioethanol
2) biohydrogen
3) biomethane
4) biodiesel

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