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Third generation biofuel

First-generation biofuels cannot compensate for the biodiesel production using vegetable oils or other oil crops. This lack has prompted the research focus towards the use of oleaginous microbial biomass such as algae, yeast, and bacteria as feedstocks for biodiesel production. Oleaginous microbial biomass is characterized by a lipid content of more than 20%. These organisms can be grown at higher yields using limited resources and can be used to produce both the feedstock as well as fuel. They have a capability to accumulate large amounts of fatty acids in their biomass which can be extracted and used to produce biodiesel. Besides oils, certain species are rich in carbohydrates and proteins which can be used as feedstocks for the anaerobic digestion process for biogas or biohydrogen production. Furthermore, by using biomass biorefinery approaches, certain species can be used to generate high value products that could compensate for the cost of biofuel production.

Algal-based biofuels are the most widely studied group of third-generation biofuels. Algae are primitive photosynthetic organisms that lack roots, stems, or leaves. They assimilate atmospheric CO_2 and use light energy for their growth. In fact, they absorb CO_2 much more efficiently compared to terrestrial plants. Depending upon the cell size, they can be micro (ranging from a few micrometers to a few hundreds of micrometers) or macroscopic (extending up to 100 feet) in nature. Due to their biodiversity, a range of fuels, such as ethanol, butanol, biodiesel, biohydrogen, and biogas, can be obtained using algal feedstocks, thus projecting their versatility for biofuel production.

They can be grown in autotrophic (inorganic), heterotrophic (organic), and mixotrophic (combination of autotrophic and heterotrophic) modes and cultivated in outdoor raceway ponds or indoor photobioreactors. These different culture techniques allow the use of different combinations for obtaining high yields of biomass and bioproducts. Algae have several inherent advantages for biofuel production; for example, they can produce lipids, carbohydrates, and proteins in large quantities, they can synthesize 100 times more oil per acre of land than any other plant, they can absorb CO_2 from discharge gases, thus aiding in reduced

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GHG emissions, and they can be grown in wastewaters and saline or brackish waters rather than being dependent on the water used for irrigation or human consumption. Certain species, when grown in nutrient-limiting conditions, can convert the free fatty acids (FFA) into triacylglycerol (TAG) accounting for 20%–50% of their dry weight, which in turn can be converted to biodiesel using the transesterification process. However, the nutrient-limiting condition could affect the growth and biomass production. Thus, the selection of the most efficient algal strain with the desired composition is essential to achieve high yields of biofuels. The table in the next slide provides the carbohydrate, protein and lipid content of selected microalgal species. It can be observed that certain species such as Nanochloropsis oceanica and Chlamydomonas reinhardtii are rich in lipid contents and thus are ideal for biodiesel production. Similarly, Scenedesmus sp. and Arthrospira sp. are rich in carbohydrates and are ideal feedstocks for ethanol, biogas, or biohydrogen production via fermentative or anaerobic digestion processes.

At present, only four algal strains are grown in large scale for commercial purposes. These strains include Spirulina sp. and Chlorella vulgaris, which are cultivated in large scale for their biomass which is used in food and health industries, and Dunaliella salina and Haemotococcus pluvialis, which are cultivated to extract carotenoids (like beta-carotene and astaxanthin) for use as health supplements. However, the use of algal strains for biofuel production is still far from reality. Despite many technical and molecular advances, several discrepancies emerge between the projected outcomes based on extrapolations and actual experimental data. The algal oils produced are mostly in unsaturated forms that tend to be volatile at high temperatures and thus are more prone to degradation.

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Compositional Analysis of Selected Algal Biomass

Algae	Carbohydrate (g/g)	Protein (g/g)	Lipid (g/g)
Macroalgae			
Laminaria japonica	0.56	0.08	0.01
Ecklonia stolonifera	0.48	0.13	0.02
Undaria pinnatifida	0.40	0.15	0.02
Hijikia fusiforme	0.28	0.05	0.01
Gelidium amansi	0.61	0.16	0.03
Porphyra tenera	0.35	0.38	0.04
Gracilaria verrucosa	0.33	0.15	0.03
Codium fragile	0.32	0.10	0.01
Microalgae			
Chlorella vulgaris	0.38	0.49	0.007
Chlorella sp.	0.25	0.41	0.11
Chlorella sorokiniana	0.14	0.14	
Nanochloropsis oceanica	0.33	0.10	0.34
Chlamydomonas reinhardtii	0.17	0.48	0.21
Porphyridium cruentum	0.40–0.57	0.28–0.39	0.09–0.14
Dunaliella bioculata	0.04	0.49	0.08
Tetraselmis maculata	0.15	0.52	0.03
Prymnesium parvum	0.25–0.33	0.28–0.45	0.22–0.38
Scendesmus quadricauda	0.23	0.47	0.019
Scendesmus dimorphous	0.21–0.52	0.08–0.18	0.16–0.40
Spirogyra	0.33–0.64	0.06–0.20	0.11–0.21
Scendesmus obliquus	0.10–0.17	0.50–0.56	0.12–0.14
Chlorella vulgaris	0.12–0.17	0.51–0.58	0.14–0.22
Spirulina maxima	0.13–0.16	0.60–0.71	0.06–0.07
Spirulina platenensis	0.08–0.14	0.46–0.63	0.04–0.09
Anabaena cylindrica	0.25–0.30	0.43–0.56	0.04–0.07
Synechococcus sp.	0.15	0.63	0.11
Dunaliella saliana	0.32	0.57	0.06
Chlorella pyrenoidosa	0.26	0.57	0.02
Euglena gracilis	0.14–0.18	0.39–0.61	0.14–0.20
Arthrospira platensis	0.44	0.45	

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Cultivation in open ponds is another major hurdle because it leads to cross contamination and seasonal variation issues that must be carefully addressed. The concept of an algal biorefinery to generate multifaceted products from a single species is presumptuous because it is impossible for a single strain to have all the necessary properties. Although genetic modification to induce desirable properties is possible, the economic viability and the environmental impacts of such species are questionable. Major research and development in this area is focused on redirecting metabolic pathways to generate the desired products without compromising growth, development of cost-effective bioreactors, coproduction of high-value chemicals, and the optimization of the harvesting or extraction process to minimize the recovery costs. These developments could make the algal biofuel production profitable in the near future. The life cycle analysis of algal-based biofuels show that it would take more than 25 years for this technology to be commercially available.

In addition to algae, however, yeast, filamentous fungi, and bacteria also have the tendency to accumulate oils at high concentration. These organisms are capable of metabolizing a diverse range of carbohydrates and can grow at faster rates with higher cell densities compared to algal cells. Bacteria are the most fast-growing and easily cultivable organisms. Only a few species belonging to the group actinomycetes have the capability to accumulate lipid in the form of polyhydroxyalkanoate (PHA). Although a few studies have been reported for extraction of PHA for biopolymer production, no study has been conducted for using these organisms for biodiesel production. Yeasts and filamentous fungi (molds) on the other hand have been used to produce oleochemicals such as fuels, chemicals, food, and feed ingredients for several decades. The commercial production of these oils demonstrates the feasibility of using yeasts and filamentous fungi for biodiesel production. Yeasts can store up to 70% weight per weight (% weight/weight) lipid in various forms such as triacylglycerides, diacylglycerides, monoacylglycerides, fatty acids, sterol esters, glycolipids, and polyprenols.

At present, a few species such as Rhodosporidium toruloids, and Trochosporon pullulans have been reported for biodiesel production and several new species continue to be discovered. The fatty acid profile and the storage lipid are bound to be varied depending on the type of species. So, like microalgae, strain selection and strain improvement must be the primary concern for biodiesel production. At present, the excessive production costs seem to be the hurdle for this technology to reach the commercial market. At present, using this biomass to produce chemicals, food, and health care supplements seems more economically viable than for biodiesel production. However, these barriers can be overcome by developing high-yielding strains through genetic modifications with simultaneous extraction of multiple products such as vitamins, pigments, and proteins.

Fourth generation biofuel

The availability of raw materials used for first-, second-, and third-generation biofuels is geographically limited and thus is not sustainable on a global scale. The use of synthetic biology and metabolic engineering tools to derive biofuels from sunlight and CO_2 as the inexhaustible and inexpensive resources has been categorized as fourth-generation biofuels. The intent of fourth-generation biofuels is to avoid biomass destruction with complete capturing and storing of CO_2 to make the process carbon neutral or carbon negative. By applying this technology, new artificial biological systems can be developed or the metabolic pathways in the existing biological systems can be reconstructed to generate high quality biofuels. These fourth-generation biofuels can be categorized as solar biofuels, electrobiofuels, and synthetic biofuels depending upon the nature of the raw materials.

The solar biofuels are obtained by the genetic modification of the existing photosynthetic organisms such as unicellular microalgae or cyanobacteria to produce the desired fuels. Not only hydrogen can be generated by the photosynthetic water splitting but also reduced carbon compounds such as methane or ethanol can be extracted using CO_2 as raw material. The production of solar biofuels requires careful understanding of the natural light harvesting mechanisms, the associated enzymes, and the means of carbon metabolism of the photosynthetic

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organisms to alter the pathways for generating valuable chemicals (that are currently obtained via fossil fuels) and biofuels. Successful demonstrations have already been performed using cyanobacteria by introducing various fermentative metabolic pathways into its genome to obtain valuable products such as hydrogen, ethanol, butanol, and lactic acid. Since this technology is fast emerging, new scientific breakthroughs are expected which may be a promising solution to the world's energy crisis.

Electrofuels are obtained by the combination of photovoltaic cells or solar cells and the bioelectrochemical systems. In case of microbial electrosynthesis, reduced carbon-based chemicals and fuels can be generated using CO_2 and electrons from electrodes as carbon and energy sources, respectively. These systems are a reverse modification of microbial fuel cells wherein the microorganisms known as electrotrophs that can uptake electron from solid electrodes are used. Currently, this technology is in its nascent stage of research and only a few proof of principle studies have been carried out. Although promising results have been observed, several technical barriers must be overcome for this technology to reach the commercial market.

Synthetic biofuels are developed by microfabricating the biological system at the laboratory scale and developing new metabolic routes to obtain the product of interest. These systems require modeling and designing of the targeted biological organelles with proper simulation and standardization for the targeted products. It provides a platform to develop new synthetic biology tools (based on the principles of biology and biochemistry) to engineer. The technology, however, is still in the developmental stages and there is lot of scope to construct small synthetic factories or organelles to generate cost-efficient biofuels.

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Problems:

Q1: Multiple Choice Questions (MCQs)

1. **Third-generation biofuels are mainly produced from:**
   A) Food crops
   B) Forest residues
   C) Microorganisms
   D) Crude oil
   Answer: C)

2. **Oleaginous microorganisms contain more than:**
   A) 5% lipids
   B) 10% lipids
   C) 20% lipids
   D) 1% lipids
   Answer: C)

3. **Which organism can produce the highest oil per acre?**
   A) Corn
   B) Soybean
   C) Algae
   D) Sugarcane
   Answer: C)

4. **Ideal lipids for biodiesel in algae are stored as:**
   A) Carotenoids
   B) Triacylglycerols (TAGs)
   C) DNA
   D) Cellulose
   Answer: B)

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5. **Which of the following is NOT a challenge in algal biofuel production?**
   A) High production cost
   B) Seasonal contamination
   C) No oxygen generation
   D) Oil instability at high temperatures
   Answer: C)

6. **Which species are grown commercially for food and health supplements?**
   A) Spirulina and Chlorella
   B) Nannochloropsis and Scenedesmus
   C) Corn and soybean
   D) Saccharomyces and Clostridium
   Answer: A)

7. **Yeasts can store up to:**
   A) 10% lipid
   B) 30% lipid
   C) 70% lipid
   D) 5% lipid
   Answer: C)

8. **Major research focus in third-generation biofuels includes:**
   A) Burning fossil fuels
   B) Developing high-yielding strains
   C) Increasing food crop farming
   D) Decreasing biomass production
   Answer:B)

9. **Which of the following organisms are considered oleaginous microorganisms suitable for biodiesel production?**
   A) Algae, yeast, and bacteria
   B) Fungi, mosses, and lichens
   C) Grasses, trees, and shrubs
   D) Insects, worms, and protozoa
   Answer: A)

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10. Why are yeasts currently more useful for chemicals and food instead of biodiesel?

A) Low oil content
B) High production cost
C) No lipid accumulation
D) Cannot be cultivated
Answer: B)

11. Fourth-generation biofuels mainly rely on:

A) Food crops
B) Wood and agricultural waste
C) Sunlight and CO_2
D) Animal fats
Answer: C)

12. The goal of fourth-generation biofuels is to:

A) Produce only hydrogen
B) Increase fossil fuel usage
C) Achieve carbon-neutral or carbon-negative production
D) Use more farmland
Answer: C)

13. Solar biofuels are produced by:

A) Burning biomass
B) Genetically engineering photosynthetic microorganisms
C) Fermenting sugarcane
D) Extracting oil from seeds
Answer: B)

14. Cyanobacteria can be engineered to produce:

A) Plastic
B) Metals
C) Hydrogen, ethanol, and lactic acid
D) Coal
Answer: C)

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15. Electrofuel production uses:

A) Electrotrophs that absorb electrons
B) Only sunlight
C) Natural gas
D) Fossil carbon
Answer: A)

16.Which system is the reverse of microbial fuel cells?

A) Transesterification
B) Microbial electrosynthesis
C) Fermentation
D) Natural photosynthesis
Answer: B)

17.Synthetic biofuels are produced by:

A) Growing plants faster
B) Designing artificial biological systems with new metabolic pathways
C) Using crude oil
D) Burning algae
Answer: B)

18.A major challenge with electrofuels is:

A) Lack of microorganisms
B) High energy density
C) Early research stage and technical barriers
D) Too much commercial production
Answer: C)

19.Solar biofuel production requires understanding:

A) Astronomy
B) Photosynthetic light harvesting and carbon metabolism
C) Deep ocean mining
D) Fossil fuel refining
Answer: B)

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20. Synthetic biology enables scientists to:

A) Destroy biomass
B) Create artificial organelles to produce biofuels
C) Increase CO_2 emissions
D) Eliminate microorganisms
Answer: B)

Q2: Short Questions

1. **What are third-generation biofuels mainly produced from?**

They are produced from oleaginous microorganisms such as algae, yeast, and bacteria.

2. **What characteristic makes microbial biomass suitable for biodiesel production?**

They contain more than 20% lipids in their biomass.

3. **Enumerate two advantages of using algae for biofuel production.**

Answer:

- **Algae can produce very high oil yields—up to 100 times more oil per acre than plants.**
- **They can be grown in wastewater, saline, or non-arable land, reducing competition with food crops.**

4. **Why are algae considered highly efficient for CO_2 absorption?**

Because they assimilate CO_2 faster than terrestrial plants through photosynthesis.

5. **What types of fuels can be produced from algae?**

Ethanol, butanol, biodiesel, biohydrogen, and biogas.

6. **What are photobioreactors used for?**

For cultivating algae under controlled indoor conditions to increase biomass productivity.

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7. Why is strain selection important in algal biodiesel production?
Because different algal species contain different lipid, carbohydrate, and protein levels.

8. Why is commercial-scale algal biofuel production still limited?
Due to high production costs, contamination issues, and unsaturated oil instability at high temperatures.

9. Which microalgae are rich in lipids and ideal for biodiesel?
Nannochloropsis oceanica and Chlamydomonas reinhardtii.

10. Besides algae, which microorganisms can accumulate large amounts of oil?
Yeasts, filamentous fungi, and some bacteria.

11. What is the main obstacle in using yeasts and fungi for commercial biodiesel?
High production costs.

12. Why are algae more sustainable than plants for biofuel production?
Because they can grow in wastewater, saline water, and need less land while producing far more oil per acre.

13. Why can nutrient limitation increase biodiesel yield in algae?
Because algae convert fatty acids into triacylglycerols (TAGs), which are ideal for biodiesel.

14. Why is cultivation in open ponds risky?
Due to contamination, changing weather, and seasonal variations that reduce productivity.

15. Why is algal oil not always ideal for direct biodiesel use?
Because it is mostly unsaturated and degrades easily at high temperatures.

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16. What resources are fourth-generation biofuels mainly derived from?
They are derived from sunlight and CO_2 using synthetic biology and metabolic engineering.

17. What is the main goal of fourth-generation biofuels?
To produce carbon-neutral or carbon-negative biofuels by capturing and storing CO_2 without destroying biomass.

18. How are solar biofuels produced?
By genetically modifying photosynthetic organisms like microalgae and cyanobacteria to produce fuels directly from CO_2 and sunlight.

19. What fuels can be produced by photosynthetic water splitting?
Hydrogen, methane, ethanol, and other reduced carbon compounds.

20. What are electrobiofuels (electrofuels)?
Fuels generated from CO_2 and electrons coming from electrodes using bioelectrochemical systems.

21. What type of microorganisms are used in microbial electrosynthesis?
Electrotrophs—microorganisms that can uptake electrons from solid electrodes.

22. Why are electrofuels not commercially available yet?
Because the technology is still new and faces several technical limitations.

23. What are synthetic biofuels?
Biofuels made by constructing artificial biological systems or designing new metabolic pathways in the lab.

24. What do synthetic biofuel systems require before real-world use?
Modeling, simulation, and standardization of the engineered organelles.

25. Why is fourth-generation biofuel research important?
It offers a promising solution to the global energy crisis with cleaner and more sustainable fuel production.

26. Why can fourth-generation biofuels be carbon-negative?
Because they capture and store CO_2 while producing fuel, reducing greenhouse gases in the atmosphere.

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27. Why are genetically modified cyanobacteria suitable for solar biofuels?
They perform photosynthesis, absorb CO_2, and can be engineered to produce valuable products like hydrogen and ethanol.

28. Why is microbial electrosynthesis described as the reverse of microbial fuel cells?
Fuel cells generate electricity from microorganisms, while electrosynthesis uses electricity to produce fuels from microorganisms.

29. Why aren't synthetic biofuels commercial yet?
They are still in the developmental stage and need optimization, design, and cost-effective production systems.

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