CHAPTER 1

Introduction

1. Introduction

Life is one of the most aesthetically pleasing attributes, distinguishing planet Earth from the rest of the known universe. The sustenance of life on Earth is based upon the need for an energy source to facilitate its routine functions. In essence, life is all about energy conversion and energy balance. Almost all the lifeforms known to us, ranging from single-cell microorganisms to complex life forms like plants, animals, birds, and even humans, are directly or indirectly dependent on solar energy. From prehistoric times, life on Earth gradually evolved to sustain a balanced hydrocarbon-based energy cycle utilizing solar energy [1]. Photosynthetic organisms like plants, algae, phytoplankton, and some bacteria are the primary producers known as autotrophs. Autotrophs harness solar energy directly and store it in the form of complex organic compounds like carbohydrates, fats, proteins, etc., which are to be used when needed. Autotrophs accomplish this with the photosynthesis reaction utilizing solar energy to process simple carbon in the form of carbon dioxide and combine it with water and other nutrients to form sugars [2], as shown in Figure 1.1.

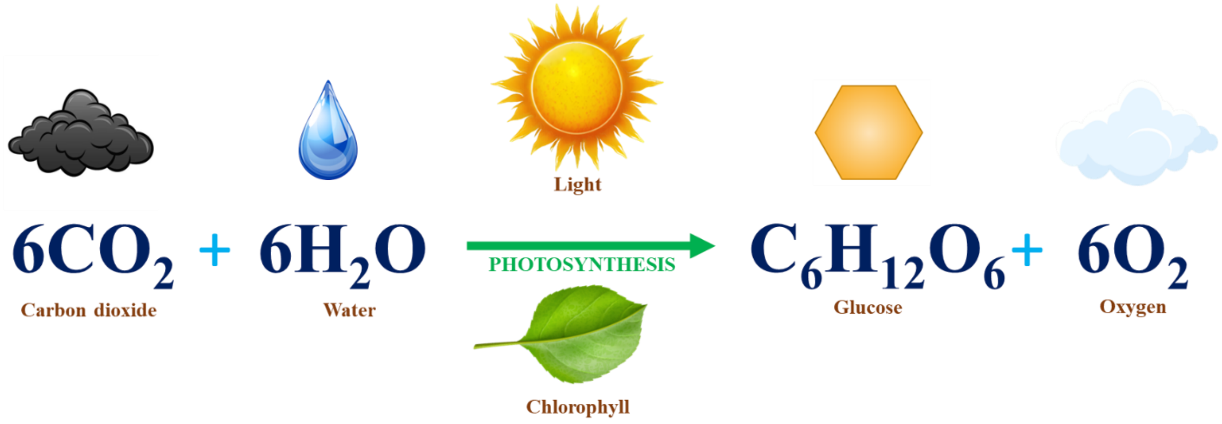


Figure 1.1: The photosynthesis reaction.

Next in the food chain comes heterotrophs that consume other lifeforms to utilize the solar energy they store. First in the heterotrophs comes the herbivores like cows, goats, squirrels, sardines, etc., that consume the autotrophs and utilize the solar energy stored by the autotrophs in the form of complex organic compounds for their development and to fulfill their energy needs. The higher forms of heterotrophs, known as primary predators like foxes, cats, seals, etc., consume the herbivores. Secondary predators, like eagles, lions, sharks, etc, eat the primary predators. And finally comes the decay detritivores that feed on the dead organisms. Detritivores like fungi and bacteria break the complex organic compounds and release them to the soil, water, and atmosphere in the form of simple compounds like carbon dioxide and nutrients to be used by the next cycle of life.

This had been the way of life on earth for millions of years until the *Homo sapiens*, or in simple terms, we humans had evolved as an intelligent species and learned to exploit more energy for our comfort and voracity than typically needed for our sustenance [3, 4]. The evolution of humans as a civilization can be marked by the use of energy in different forms and the leap ahead of other species. Initially, we learned to control fire as an energy source to cook food, keep us warm, and for other allied activities [5]. Since then, we have evolved and learned to exploit different energy sources like wind, hydro, biomass, etc., to make our lives more comfortable and progress as a species. Human evolution took an exponential leap in the early 18th century as we learned to exploit the dense energy source trapped under the Earth's crust in the form of fossil fuels. Fossil fuels are amongst the densest forms of solar energy reserve known to us having energy densities of approximately 24-34, 45, 46, and 55 MJ/kg for coal, diesel, petrol, and natural gas, respectively, compared to 10-16 MJ/kg for woody biomass [6, 7]. Fossil fuels are made of primordial flora and fauna burried in the Earth's crust for millions of years in extreme pressure and temperature. The beginning of commercial oil exploration is marked in the year 1859 by the first oil well drilled by Colonel E. L. Drake near Titusville, Pennsylvania, USA [8, 9], and oil production has exponentially increased since then, as shown in Figure 1.2, with peak production estimated to be in the early 21st century. Notably, the human population grew unprecedentedly with the commercial exploitation of fossil fuels. The effect of petroleum usage on the human population is reflected in Figure 1.3. Initially, the human population grew at a constant pace, however, with the discovery and commercial use of petroleum in the early 1900's the human population exploded. The exponential growth can be caused by different factors, such as increased food production due to the Green Revolution, which again happened due to using petroleum to produce fertilizers and mechanized farming equipment. The use of petroleum resulted in surplus food, a comfortable lifestyle, an increase in life expectancy, and countless other benefits to human society. There are different forms of energy being used by us presently, like nuclear, hydro, solar, wind, tidal, geothermal, etc.. However, due to its reliability, high energy density, ease of mobility, and many other advantages, fossil fuels are the most preferred energy source in modern human civilization, as seen in Figure 1.4.

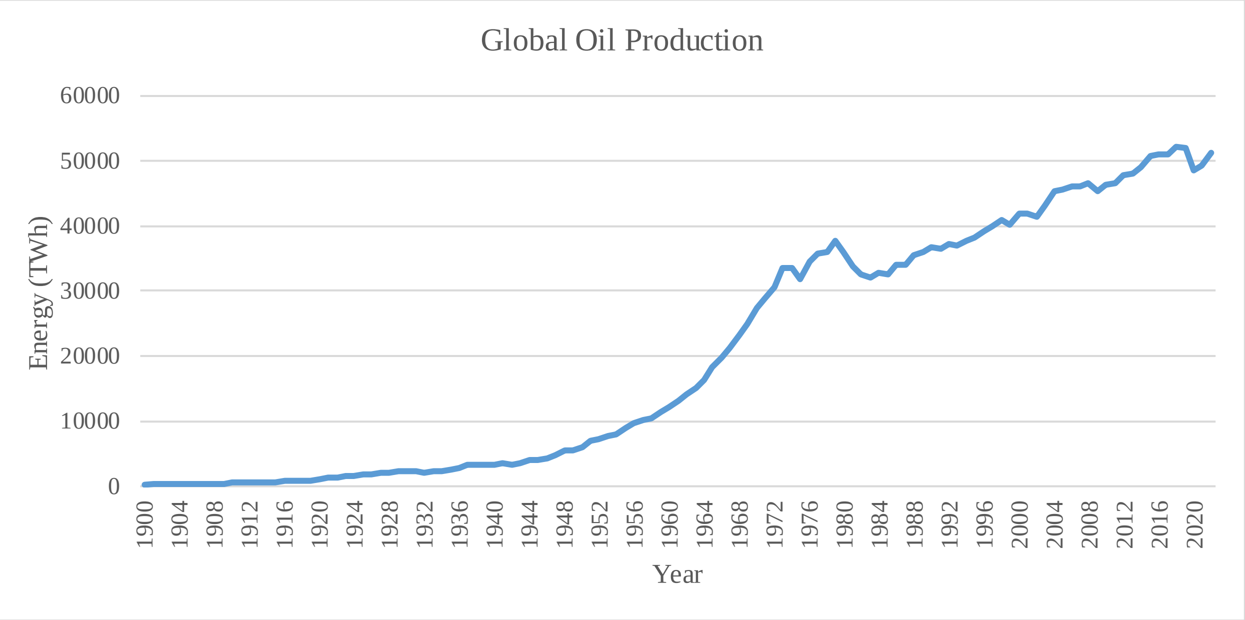


Figure 1.2: Global oil production [10].

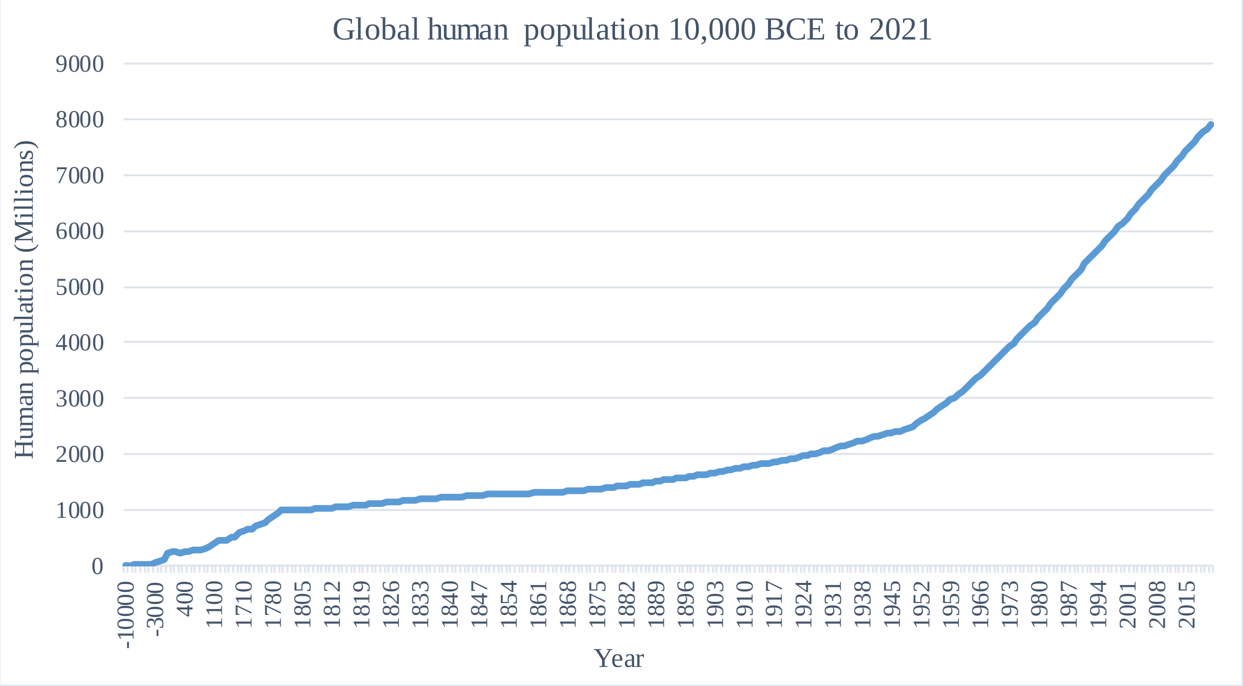


Figure 1.3: Global human population [11].

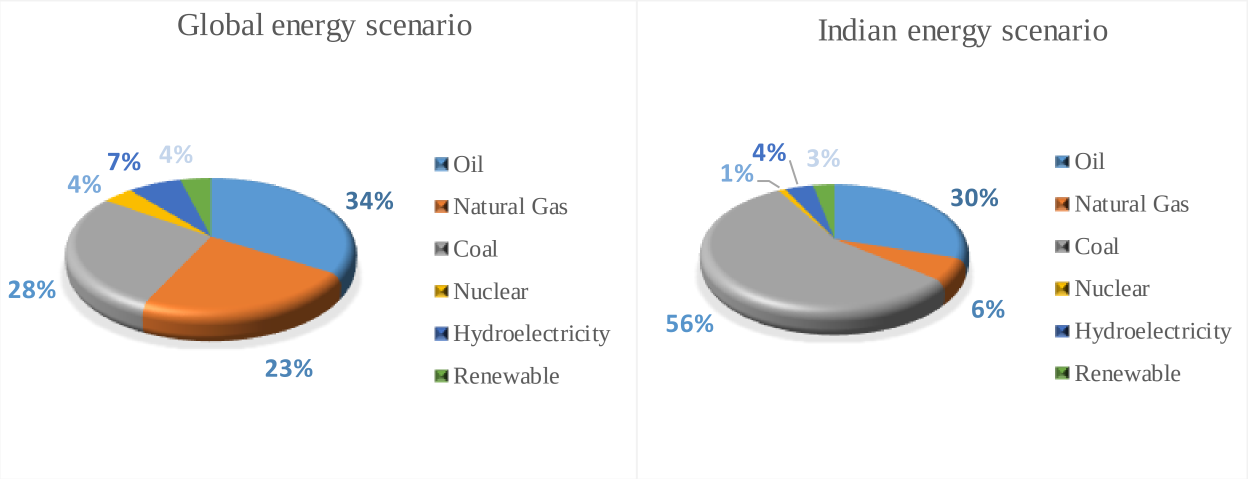


Figure 1.4: Pie chart showing the percentage of different energy sources used globally and in India to fulfill its energy needs [12].

While utilizing fossil fuels for energy production and allied applications, carbon compounds trapped within the earth's crust for millions of years in the form of fossils are mined out and released into the atmosphere as carbon dioxide. Due to the excessive exploitation of fossil fuels for our advantage since the 1900s', we have disrupted the harmony of nature and spiked the carbon dioxide levels in the atmosphere at an unprecedented level, as seen in Figure 1.5.

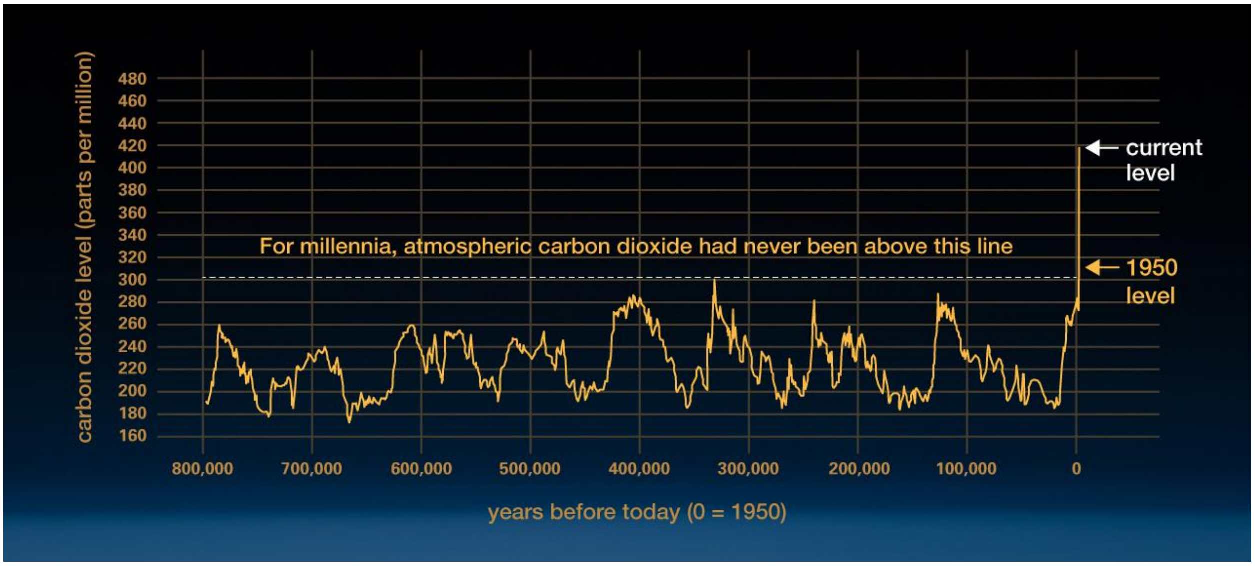


Figure 1.5: Concentration of Carbon dioxide in the air [13].

The atmospheric carbon dioxide levels spiked above 300 ppm, which had not occurred for thousands of years. Figure 1.5 shows Earth's atmospheric carbon dioxide levels during its last three glacial cycles, measured by capturing air bubbles trapped in ice sheets and glaciers. The carbon dioxide levels have been increasing ever since, and currently, as of May 2024, it is at 427 ppm, measured at Mauna Loa Observatory, Hawaii, in recent years, as shown in Figure 1.6.

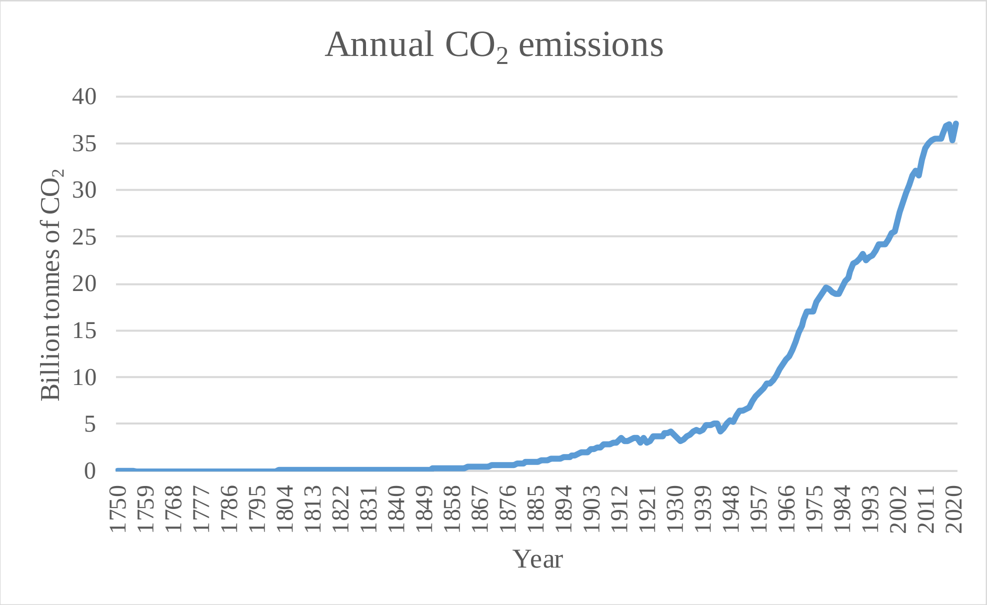


Figure 1.6: Annual CO_2 production [14].

High levels of CO_2 in the atmosphere create severe environmental concerns because CO_2 is a potent greenhouse gas, meaning the excessive quantity of CO_2 in the atmosphere causes the earth to absorb more solar radiation, warming up the earth's crust rapidly and causing the rise in global average temperature. The increase in the earth's temperature causes severe catastrophes like the melting of polar ice, rise in sea levels, climate change, abnormal weather patterns, and threatens the extinction of many species of plants and animals, to name a few. The high level of CO_2 in the atmosphere is absorbed by ocean water, causing acidification of the oceans as the atmospheric CO_2 dissolves in the ocean water to form carbonic acids (HCO_3). Acidification combined with a rise in global temperature threatens the biodiversity within the oceans around the globe [15, 16]. These issues have raised severe concerns amongst the international scientific community; hence, people are looking for alternatives to petroleum. In the past few decades, policy makers, government representatives, industry personals, and scientific communities have gathered to discuss the severances of the problems and find solutions to tackle the issues. The Montreal Protocol (1987) [17], United Nations Framework Convention on Climate Change (1992) [18], and Kyoto Protocol

(1997) [19] are examples of some of the significant events taken up internationally to prevent dangerous anthropogenic interference with the earth's climate system.

On the 12th of December 2015, at the United Nations Climate Change Conference (COP21), held in Paris, France, 196 parties ratified a legally binding international pact on climate change. The treaty, commonly known as the Paris Agreement [20], came into force on the 4th of November 2016. The main objective of the Paris Agreement is, in short, to keep the average global temperature well below 2°C over pre-industrial levels and to work toward limiting the increase in temperature to 1.5°C above pre-industrial levels. According to the United Nations Intergovernmental Panel on Climate Change, going over the 1.5°C threshold could result in more extreme climate change effects, such as more frequent heat waves, droughts, and flooding. To limit global warming to 1.5°C, greenhouse gas emissions must peak before 2025 at the latest and decline 43% by 2030.

Most recently, the United Nations Climate Change Conference (COP26) held in Glasgow, Scotland, from the 31st of October to the 13th of November 2021, brought together almost 200 countries to negotiate over two weeks and come up with the Glasgow Climate Pact [21]. Despite the fact that coal, oil, and gas are the primary sources of global warming, one of the major outcomes of the Glasgow Climate Pact was the countries' ultimate agreement to a provision that called for a phase-down of coal power and a phase-out of “inefficient” fossil fuel subsidies. These two important issues had never before been specifically addressed in decisions made at UN climate talks.

Additionally and most importantly, fossil fuels are non-renewable energy sources, and different sources have estimated that with the current utilization rate, the earth is left with petroleum reserves to sustain for roughly 50 years [22]. Figure 1.7. visualizes the quantities of proven reserves of the most widely used energy sources compared to global energy requirements and global solar energy received. It can be seen that major energy sources on which human civilization is dependent, like coal, petroleum, natural gas, nuclear fuel, etc., have limited life spans. Thus, we must replace non-renewable sources with alternative renewable energy sources before they run out. Humankind cannot stop the use of fossil fuels right away as economic growth and development are underpinned by a stable and reliable energy source. The search for a sustainable and reliable renewable alternative to petroleum is of prime importance for global society's survival and avoiding major catastrophes to human civilization.

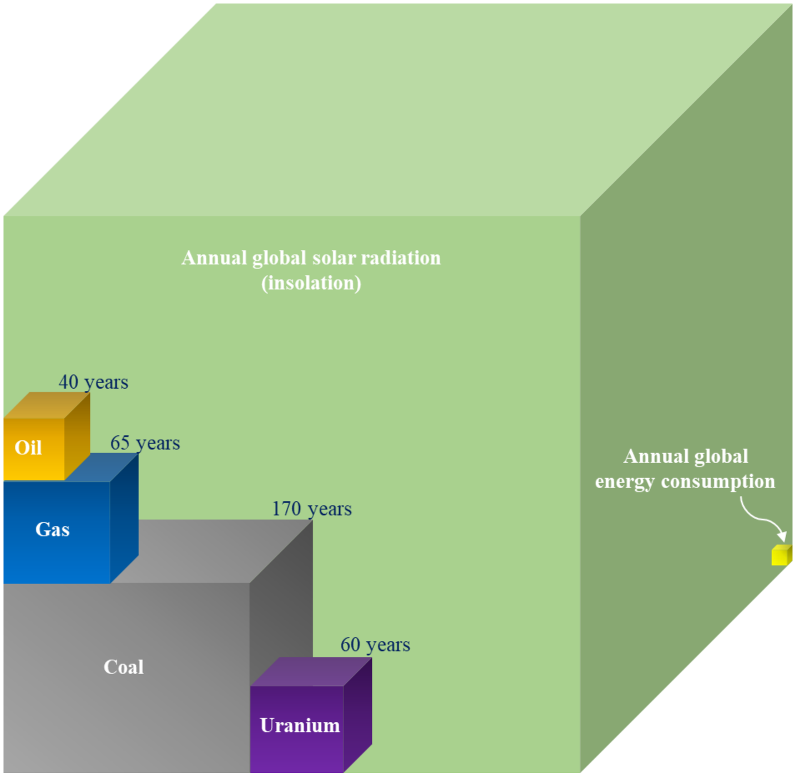


Figure 1.7: A representation of the global energy scenario and different energy sources [23].

1.1.Alternative renewable options

Different renewable energy alternatives are available, such as solar, wind, tidal, hydroelectric, biomass, etc., are being used and are under research for performance improvement. Different energy sources have distinctive characteristics, giving them a specific edge for certain applications while making them unsuitable for others. Solar energy has enormous potential as it is available in sheer abundance. One hour of solar energy received on the earth's surface is sufficient for the entire global annual energy needs [24, 25]. However, it is impossible to do so because we do not have the technology to capture and store that massive amount of energy. The solar energy received on the earth's surface is in the form of ultraviolet, visible, and infrared radiation. It needs to be concentrated or converted into other forms like electrical, thermal, chemical, or potential energy to store and use for our benefit.

Humankind has devised ingenious techniques to capture, utilize, and store renewable solar energy in different forms. Solar photovoltaic cells can capture solar energy and directly convert it to electricity, which can be stored in batteries or used directly through grid supplies [26]. Solar thermal collectors, solar concentrators, etc., capture, store, and utilize solar energy in the form of heat for process heating or to generate steam to produce electricity [24]. Solar thermal energy can be stored in molten salts or other media with high specific heat [27]. We also indirectly capture and store solar energy in the form of potential energy by building dams and using them to drive hydroelectric turbines and generate electricity. Solar energy is also indirectly captured through kinetic energy using wind turbines to generate electricity. Biomass is another form of indirect solar energy captured and stored in the form of chemical energy and used to produce biofuels. Solar energy has also been used to break water molecules through electrolysis to produce green hydrogen [28]. Hydrogen is then stored and used to produce heat by burning it directly or producing electricity using fuel cells. Apart from solar-based renewable energy sources, there are a few other forms, such as tidal energy [29] and geothermal energy [30]. All forms of renewable energy have distinctive advantages over fossil fuels but have certain limitations, making it difficult to compete with fossil fuels. Table 1 sheds light on different forms of renewable energy sources in detail.

Table 1.1: Some of the prominent renewable energy technologies available at present.

Energy source	Description	Advantages	Disadvantages
Solar Photovoltaic	Solar photovoltaic panels are semiconductors that directly convert incident light, typically sunlight, into electricity.	Solar energy is abundant, and with the recent advances in semiconductor technology, PV is becoming more efficient and affordable.	The energy varies depending on the weather and season. Most importantly, it is unavailable at night and thus requires colossal storage capacity.
Flat plate solar thermal collectors	Solar thermal collectors are devices that collect solar heat from infrared radiation and efficiently transfer it to a working medium like water or air.	Solar thermal collectors have much higher efficiency [31] than solar PV, making them more suitable for thermal applications like heating and drying.	They are not suitable for many applications where very high temperature is required. The energy storage cost is high in this type of system.

Energy source	Description	Advantages	Disadvantages
Solar concentrators	Solar concentrators herness the solar radiations from a larger area and focuses it onto a reduced area with the working fluid or surface to increase the substance's temperature by many folds.	They are good at compensating for the intermittency of other renewable energy sources. Suitable for process heating in industries with high-temperature requirements.	They can be installed on utility scales and not on household levels. The capital cost is too high. They also possess environmental concerns.
Hydroelectricity	Hydroelectricity converts the potential energy of stored water in dams to run turbines and generate electricity.	They are reliable sources where the output can be easily adjusted depending on the load by controlling the water flow rate. They are generally safe and don't emit any pollution.	Building dams for hydroelectricity affects the area's environment and natural ecosystem. As well as it also interferes with the river's natural flow, affecting the downstream areas.
Wind energy	Wind energy herness the kinetic energy of moving air using wind turbines to generate electricity.	It is one of the cleanest forms of energy, and with the latest technological advances, it is very efficient.	Wind energy suffers from intermittency as wind is not constant at all times and year-round. In some instances, they are reported to be harmful to birds and a bit noisy.
Biofuel	Biofuels are biomass-based energy sources like ethanol, biodiesel, methane, etc., derived from plant or animal biomass that are burned to produce heat.	Biofuels have similar properties to petroleum fuels and can be used in existing petroleum-based infrastructures. Therefore, biofuels can replace petroleum-based fuels altogether.	Feedstock availability is a significant issue with biofuels. Certain issues like food and energy conflict, deforestation, and biodiversity loss are concerns with current biofuel technologies.

Energy source	Description	Advantages	Disadvantages
Hydrogen	Renewable hydrogen is generated by splitting water through the process of electrolysis using some form of renewable energy like sole PV or wind energy, etc.	Hydrogen is a clean, efficient, reliable, and bountiful energy source. Hydrogen can be produced sustainably by splitting water using renewable energy sources through electrolysis.	Storage and transport of hydrogen is a challenging process as the density is very low, and it needs to be stored at high pressure. It is also potentially hazardous due to its very high volatility.
Tidal	Tidal energy systems utilize the rising and receding sea levels caused by the tidal forces to store the ocean water in dams and run hydro turbines to produce electricity.	It has a high energy output and doesn't emit any carbon. It is a highly predictable energy source and, thus, highly reliable. Its operational and maintenance costs are also minimal.	Limited installation sites as they require a specific geographical location. The installation cost is high and has specific environmental impacts similar to wind turbines.
Geothermal	Geothermal energy systems exploit the heat within the Earth's crust to produce steam and run turbines to produce electricity or directly for process heating purposes.	It is a sustainable, environmentally friendly, and reliable energy source. It is suitable for process heating as well as electricity generation.	Its installation is restricted to certain locations with specific geographical characteristics. The cost of production is comparatively high.
Batteries	Batteries store electrical energy in the form of electrochemical potential that can be reversible or irreversible based on the technology used.	Batteries are efficient, portable, and reliable. They can store energy from renewable sources, eliminating latency issues most renewable energy systems face.	They have a limited lifespan, even for rechargeable batteries, and are costly. Rechargeable batteries require rare earth metals, making scalability a major issue.
Supercapacitors	Supercapacitors store electrical energy as static electricity that can power electrical devices.	Supercapacitors have longer lifecycles, can handle high load currents, and require less charging time than batteries.	Supercapacitors have low specific energy and a high self-discharging rate, and the cost per W is higher than batteries.

Amongst all the available renewable energy options, biofuels are one of the most promising fuel sources capable of replacing petroleum [32]. This is because biofuel has physiochemical properties and energy density similar to petroleum fuels, and existing industrial infrastructure can easily switch to biofuels. Biofuels can be described as biomass-based reservoirs of solar energy. Biofuels are derived from biomass feedstock, which has been refined via the natural biotic ecosystem's extensive evolutionary process spanning millions of years.

With decades of research and development, biofuel technologies have evolved significantly to become industrial-grade fuels and are becoming much more important as a substitute for petroleum [33]. However, with the current state of the art, producing biofuels at the scale and cost needed to substitute petroleum, let alone replace it substantially, does not seem possible [34]. This research is conducted to investigate and find solutions for biofuels' issues in being a sustainable and widely accepted energy source.

1.2 Biofuels

Biofuels are fuels produced from renewable biomass (plants & animals) sources, such as crops, agricultural and forestry residues, waste materials, and algae. Biofuels are considered a cleaner and more sustainable alternative to fossil fuels because they are made from renewable sources and emit fewer greenhouse gases and pollutants when burned [35, 36]. Additionally, carbon dioxide released to the environment while burning biofuels is absorbed back during biomass feedstock production, making biofuels carbon neutral. Biofuels can be classified into different products based on their physiochemical properties, like bioethanol, biodiesel, bio-jet (SAF), biochar, and biogas.

1.2.1 Bioethanol

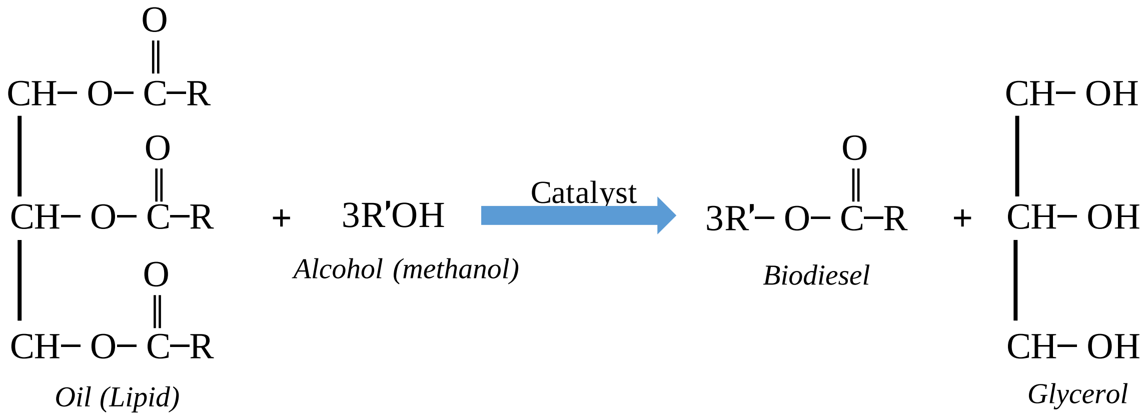
Maize, sugarcane, and grain are crops that are used to produce bioethanol by fermenting the sugars and starches present in them. Bioethanol has similar properties to petrol and thus can be substituted or blended with gasoline and used in petrol-based engines. Bioethanol has a high octane number of around 108 and has higher oxygen content than fossil-based petrol, preventing engine knocking and cleaner combustion [36]. Bioethanol is made from glucose using yeast through fermentation, as shown in Scheme 1.1. However, certain feedstock preprocessing might be required to make the feedstock suitable for fermentation during bioethanol production [36, 37]. The preprocessing varies depending on the feedstock used. With recent technological advances, bioethanol is being made from lignocellulose woody biomass feedstocks.

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{\textit{Yeast}} \underset{\text{Ethanol}}{2C_2H_5OH} + \underset{\text{Carbon dioxide}}{2CO_2}$$

Scheme 1.1: Fermentation reaction

1.2.2 Biodiesel

Biodiesel is a renewable fuel with similar properties to petroleum diesel and is derived mainly from plant-based oils or animal fats by transesterification [38, 39]. Biodiesel is typically a mono-alkyl ester derived from the long-chain fatty acids present in plant oils or animal fats. The transesterification reaction is carried out between fatty acids and methanol along with a base or acid catalyst to produce biodiesel. In the transesterification reaction, the organic functional group R" of the fatty acid is replaced with the organic functional group R' of the alcohol to produce the mono-alkyl ester and glycerol. Scheme 1.2 shows the transesterification reaction used for biodiesel production. In biodiesel production, methanol is preferred as the alcohol to react with the fatty acids; thus, the final product, the biodiesel, is also known as Fatty Acid Methyl Ester or FAME. The property of the biodiesel depends on the fatty acid used, and high-quality biodiesel is defined by compliance with the standard set by different regions of its application, like ASTM D 6751 for the United States, EN14214 for Europe, and likewise [40].



Scheme 1.2: Transesterification reaction

1.2.3 Bio-Jet (SAF)

Bio-Jet or Sustainable Aviation Fuels (SAF) are biomass derived fuels that can be used by the aviation industry to power airplanes [41]. Bio-jets are gaining strategic importance in the aviation industry as it minimizes the operational costs and curbs greenhouse gas emissions [41]. This has led to researchers from various domains like aviation industries, government agencies, biofuel companies etc. are looking for development of suitable SAF [41]. Development and utilization of SAF in a commercially viable and sustainable manner involves various factors like development pathways, feedstocks used, fuel properties, certifications etc. Technologies like Fischer-Tropsch synthesis, hydrothermal liquefaction, and hydrogenated esters and fatty acids are found to be promising pathways for development of SAF [42-44]. Microalgae as a feedstock for bio-jet production is reported to be very promising [44]. In recent times, microalgae based bio-jets are being produced and tested in airplanes [43]. Microalgae biodiesel can be converted to bio-jet fuel by hydroprocessing with Ni and zeolites catalysts [44]. Other important pathways like gasification with Fischer-Tropsch and sugar-to-jet are promising alternative process to convert microalgae to bio-jet fuel [44]. One important parameter for successful development and utilization of bio-jet or the SAF is its needs to satisfy compliance with the American Society for Testing and Materials D7566-18 standards [45].

1.2.4 Biochar

Biochar is the solid product obtained from biomass processed at high temperatures in an oxygen-deficient environment. In standard terms, biochar is also known as charcoal [46]. Biochar is typically produced by thermal decomposition of biomass with a process known as pyrolysis. The biochar production process and the properties of biochar are primarily dependent on the biomass used and the pyrolysis temperature. Thermal treatment releases water vapor and volatile matter from the biomass, leaving behind the solid fraction known as fixed carbon. At lower temperatures, the volatile gases polymerize, resulting in a higher yield of solid carbon contents [46]. Low-temperature pyrolysis in the range of 200-300 °C is called torrefaction, and it is done to retain most of the energy present in biomass in solid form. However, to achieve high purity with carbon content over 95%, biomass pyrolysis needs to be done at a temperature in the range of 1000 °C. This is done mainly with hard woody biomass. Pyrolysis at these elevated temperatures, however, causes problems in the case of agricultural residues and other materials having low ass melting temperatures. Therefore, such materials are not treated beyond 700 °C. In traditional charcoal production using charcoal pit, the temperature is in the range of 500 °C [46].

Biochar has a wide range of energy applications, from direct combustion for thermal energy generation for space heating and cooking to cofiring in coal power plants for electricity generation [47]. More advanced and diversified biochar applications on the energy front have been explored recently, such as hydrogen storage, hydrogen production, oxygen electrocatalysts, fuel cell development, supercapacitors, and lithium/sodium batteries [48].

1.2.5 Biogas

Biogas is a gaseous biofuel constituting a mixture of methane and carbon dioxide as the major fraction and other gases like nitrogen, oxygen, ammonia, etc. in trace quantities [49]. Anaerobic digestion of organic matter, including wastewater, garbage, and agricultural waste, produces biogas. Unlike bioethanol, biodiesel, or charcoal, biogas is a gaseous biofuel at normal temperature and pressure (NTP). One major advantage of biogas is that it can be produced from a diverse range of feedstock like landfills, municipal solid and liquid wastes, kitchen waste, agricultural wastes, animal manure, and energy crops [50]. Raw biogas generated by the transformation of biological matter in anaerobic conditions consists of 40-65% v/v methane and 35-55% v/v CO_2 and minor concentrations of other compositions like hydrogen sulfide, water vapor, and other gases [51]. Thus, raw biogas needs to be cleaned and upgraded before utilization to remove the CO_2, water vapor and other trace gases, which is one of the significant limitations of biogas technology. The upgradation is also essential to improve the calorific value (CV) of biogas, which is in the range of 21.5 $MJ/m^{3,}$ which is much lower in comparison to natural gas, having a CV of 35.8 MJ/m^3, and this low CV is primarily due to the presence of the CO_2 [51]. Some of the significant biogas upgradation methods are chemical scrubbing, water scrubbing, organic physical scrubbing, membrane separation, and cryogenic separation [50]. Biogas has numerous uses, ranging from space heating to process heating; it is used in diesel engines for transportation and in gas turbines for electricity generation [50, 52]. Biogas is gaining importance in the present time as one of the promising energy sources for our energy transition towards renewables.

1.3 Biofuel characterization based on feedstock

However, the most important characterization of biofuels is done based on the feedstock used to produce them. Based on the feedstock, biofuels are characterized into first, second, third, and fourth-generation biofuels. The first-generation biofuels are derived from edible biomass sources like palm, sunflower, rapeseed, soybean, corn, sugarcane, animal fats, etc. The second-generation biofuels are made from non-edible sources like Jatropha, Indian Rose Chestnut (Nahor), agriculture waste, forest waste, etc. Third-generation biofuels are those made using microalgae as the feedstock. The fourth generation of biofuels is likewise made from microalgae, but in order to improve carbon capture, biomass productivity, and lipid production, the microalgae species are genetically modified. [53].

Now, looking at the current global biofuel scenario, in 2021, the United States (US) was the leading producer of biofuel, followed by Brazil, Indonesia, China, and Germany, producing 1435.8, 839.5, 311.9, 142.7, and 121.2 Petajoules of energy from biofuels respectively [54]. Figure 1.8. illustrates some of the major biofuels produced worldwide since 2016 [55].

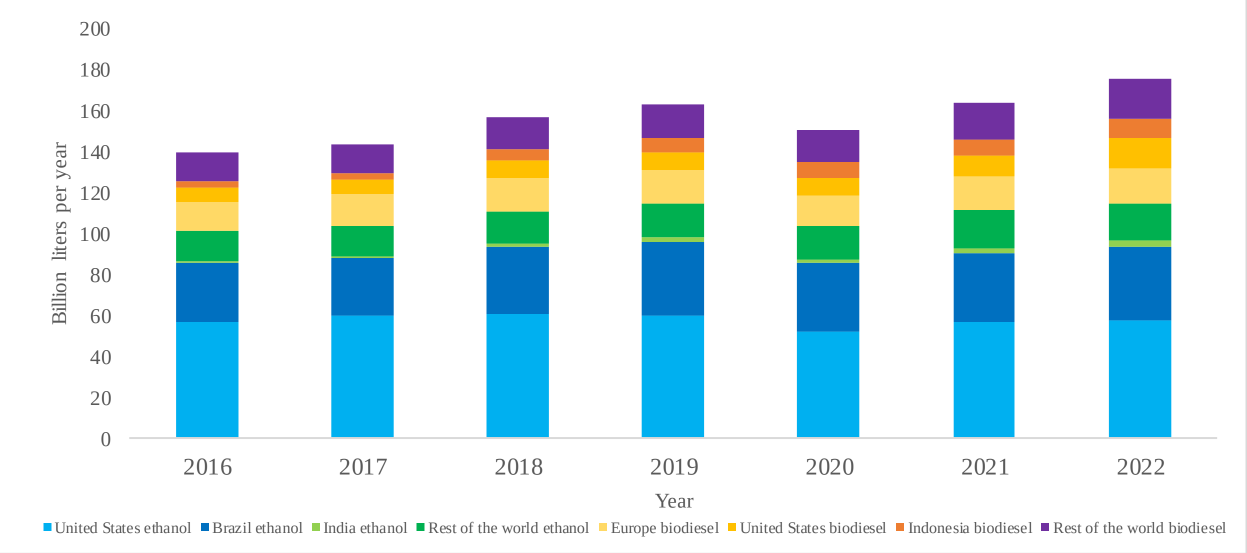


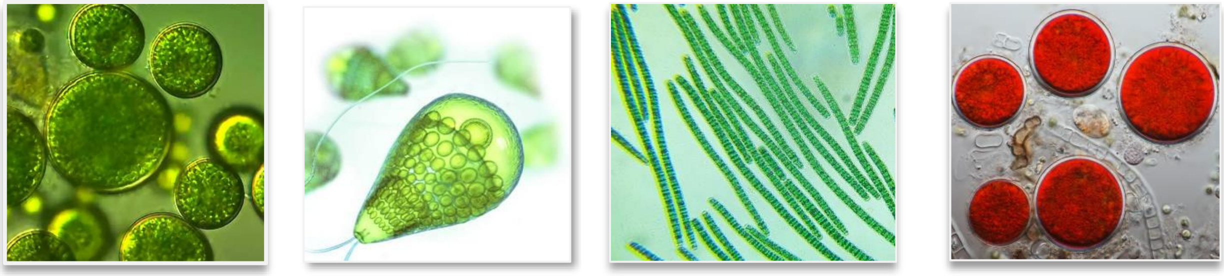
Figure 1.8: Primary biofuel production by country/region and fuel type, 2016-2022 [55].

Most biofuels produced industrially are 1st generation biofuels produced mainly from corn, sugarcane, palm, and soybean [56]. However, there are concerns about the environmental impact of producing biofuels using first- and second-generation feedstock, such as land-use changes, water use, and competition with food production. This has led researchers to focus on the 3rd and the 4th generation of biofuels using microalgae as the feedstock.

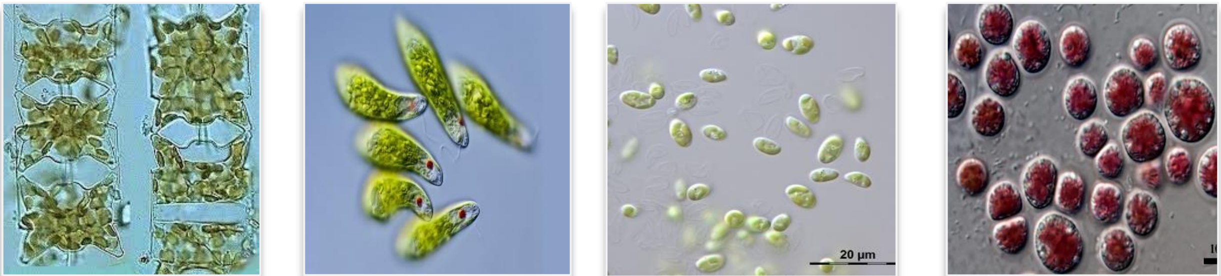
1.4 Microalgae

Microalgae are unicellular photosynthetic microorganisms living in aqueous environments. In their natural habitat, microalgae use solar energy to convert CO_2, water, and other essential minerals to microalgae biomass, which consists of lipids, proteins, carbohydrates, vitamins, and pigments [57]. There are numerous microalgae species, each with its unique characteristics. Microalgae have high photosynthesis efficiency, almost 50 times higher than some land-based plants [58], resulting in very high productivity. In optimal conditions, microalgae double its biomass under 24 hours [59]. Microalgae can accumulate high lipid contents within its cells, up to 80% of its dry weight in certain species [58]. The capacity of microalgae to accumulate a substantial amount of lipids makes them an appealing feedstock for the generation of biodiesel. Apart from lipids, microalgae also produce high amounts of carbohydrates [60], proteins [61, 62], essential pigments [61], carotenoids [63] etc. Microalgae prosper in a varied range of environments, such as freshwater, saline water, sewage, etc. All these properties make microalgae a preferred feedstock for biofuel in a bio-refinery approach. Chisti (2007) [32] reported that microalgae are the only biofuel feedstock capable of replacing petroleum-based transportation fuel.

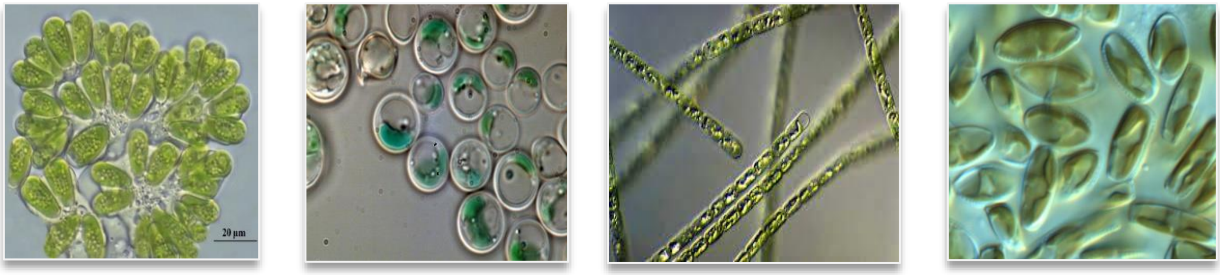
As per estimations, about 200,000-800,000 microalgae species exist, and only around 40,000-50,000 out of those have been documented [61, 64]. Due to its diverse species and unique properties, microalgae are important in various fields. Some well-documented species of microalgae are shown in Figure 1.9.



Chlorella vulgaris [65] *Dunaliella salina* [66] *Spirulina platensis* [67] *Haematococcus pluvialis* [68]



Odontella aurita [69] *Euglena gracilis* [70] *Monodus subterraneus* [71] *Porphyridium cruentum* [72]



Botryococcus braunii [73] *Galdieria sulphuraria* [74] *Aphanizomenon flosaquae* [75] *Nitzchia laevis* [76]

Figure 1.9: Some well documented microalgae species having commercial significance.

Some microalgae species' ability to accumulate high amounts of lipid makes microalgae important in the field of biofuels. Lipids accumulated by microalgae can be processed to produce biodiesel and used to replace petroleum diesel [32, 77]. High lipid content combined with fast growth rates makes microalgae a superior biofuel feedstock compared to any other feedstock available. Table 2 exemplifies the comparison between different biofuel feedstock and their productivity in terms of land usage.

Table 1.2: Biodiesel feedstock and their productivity [78, 79].

Feedstock	Lipid content (% of oil in dry biomass)	Lipid yield (L/ha/Year)	Area use (m^2 year/kg biodiesel)	Biodiesel productivity (Kg/ha/year)
Cannabis (*Cannabis sativa L.*)	33	363	31	321
Soya (*Glycine max L.*)	18	636	18	562
Jatropha (*Jatropha curcas L.*)	28	741	15	656
Camelina (*Camelina sativa L.*)	42	915	12	809
Canola (*Brassica napus L.*)	41	974	12	862
Sunflower (*Helianthus annuus L.*)	40	1070	11	946
Castor (*Ricinus communis*)	48	1307	9	1156
Palm oil (*Elaeis guineensis*)	36	5366	2	4747
Microalgae (low oil content)	30	58,700	0.2	51,927
Microalgae (medium oil content)	50	97,800	0.1	86,515
Microalgae (high oil content)	70	136,900	0.1	121,104

As mentioned earlier, the characteristics of microalgae are species-dependent. Thus, for a microalgae species to be suitable for biofuel applications, the species should have characteristics like high growth rate [80, 81], high lipid content for biodiesel application [82-84], high carbohydrate content for bioethanol and biogas application [60, 85], high tolerance to contamination and culture conditions for being suitable for mass cultivation [86, 87]. Thus, strain selection is one major step in microalgae biofuel applications. A few microalgae species with high lipid contents are listed in Table 1.3, which makes them desirable for the generation of biodiesel.

Table 1.3: Microalgae species with high lipid content

Microalga	Oil content (% dry wt.)	Reference
Botryococcus braunii	25–75	[32]
Haematococcus pluvialis	55.84	[88]
Chlorella emersonii	63	[89]
Chlorella minutissima	57	[89]
Chlorella vulgaris	56.6	[90]
Chlorella protothecoides	23	[89]
Chlorella sorokiniana	22	[89]
Crypthecodinium cohnii	20	[32]
Cylindrotheca sp.	16–37	[32]
Dunaliella primolecta	23	[32]
Isochrysis sp.	25–33	[32]
Monallanthus salina	>20	[32]
Monodus subterraneus	39.3	[91]
Nannochloris sp.	20–35	[32]
Nannochloropsis sp.	31–68	[32]
Neochloris oleoabundans	35–54	[32]
Nitzschia laevis	69.1	[92]
Nitzschia sp.	45–47	[32]
Parietochloris incisa	62	[93]
Phaeodactylum tricornutum	20–30	[32]
Schizochytrium sp.	50–77	[32]
Tetraselmis sueica	15–23	[32]
Chlorella sp.	28–32	[32]

1.5 Microalgae biofuel

Fast growth rate combined with its ability to accumulate high quantities of polysaccharide (sugars) and triacyl glycerides (fats) that can be converted into bioethanol and biodiesel, respectively, makes microalgae one of the most attractive biofuel feedstock [94]. According to life cycle analysis, microalgae biofuel can displace fossil fuels and is a chief renewable energy option for sustainable development [78]. Microalgae don't have the limitations as such of those present in oil crops and lignocellulose-based biofuels. They don't need new lands, consume much less water, and reduce atmospheric CO_2. Another significant advantage of microalgae biomass in terms of biofuel production is that it can be processed into different products as required. Figure. 1.10. illustrates various pathways followed by microalgae biofuel production and other products thus developed. Amongst all the biofuels, biodiesel, bioethanol, biohydrogen, and biogas play a vital role in the energy transition from petroleum fuels [95, 96].

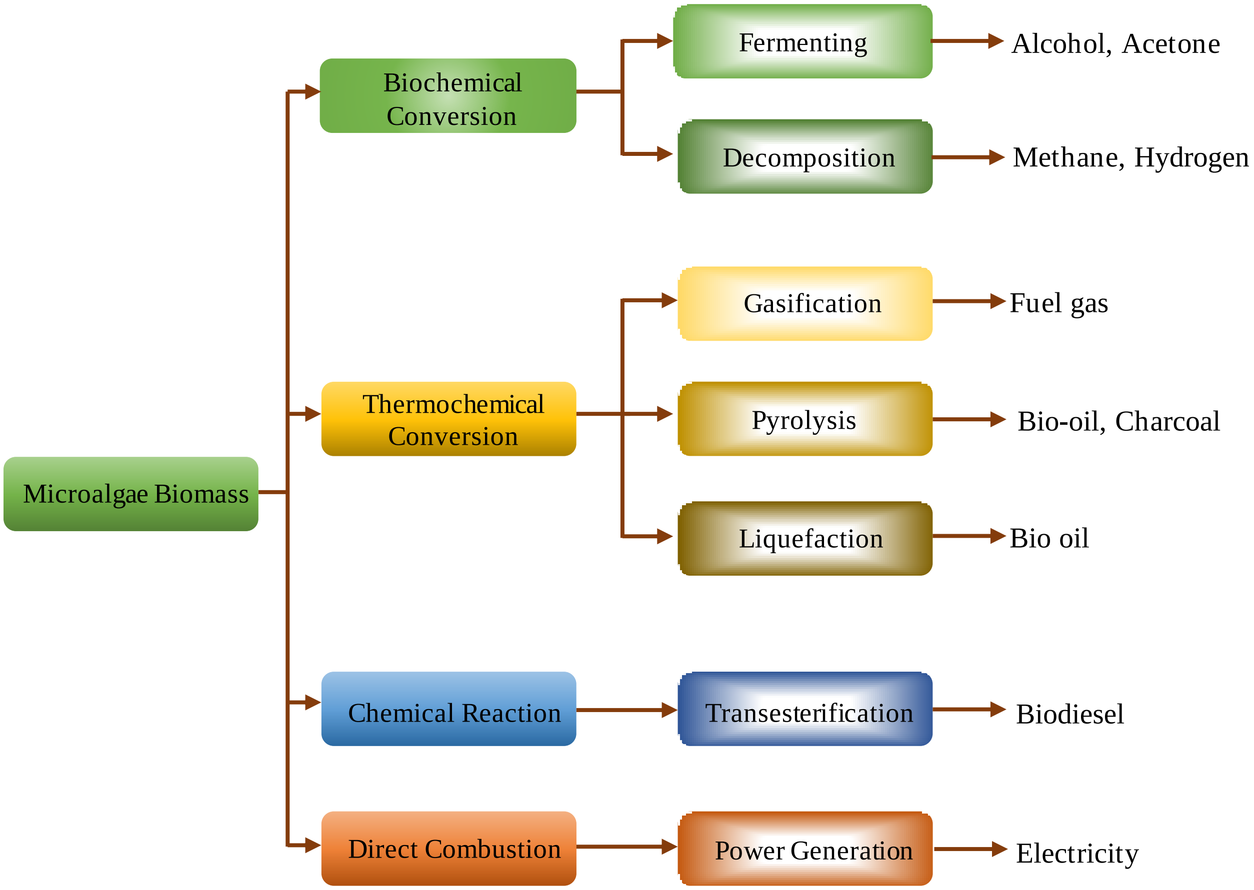


Figure 1.10: Microalgae biofuel technologies: various pathways and associated biofuels.

However, the high production cost of microalgae biomass makes microalgal biofuel technologies unacceptable [97]. Over 90% of microalgae biomass is produced globally in open culture systems [98]. Because of the low density of biomass in open culture systems, resulting in expensive processing costs, commercial microalgae biodiesel production is still unsustainable [99]. Designing sophisticated photobioreactors and creating cost-effective biomass harvesting, drying, and oil extraction procedures can make microalgae biodiesel production viable. Genetic engineering to control stress from the environment and metabolic engineering to produce elevated amounts of triglycerides are two further methods to boost commercial productivity [78].

Amongst all the steps involved in microalgae biofuel technology, microalgae biomass production is one of the most cost-intensive processes [100]. Research in the field of microalgae culture systems is crucial for advancing the sustainability of microalgae biofuel production. Developing efficient and economically viable microalgae biofuel production systems requires a deep understanding of the intricate biological and environmental factors influencing microalgae cultivation. Scholars are presently investigating novel approaches to culture and refining growing environments. In order to fully utilize microalgae as a sustainable and eco-friendly energy source, research in this area is crucial for resolving issues related to scalability, affordability, and environmental impact.

1.6 Microalgae cultivation systems

Systems for growing microalgae can be broadly divided into two categories: open- and closed-culture systems. Open systems like raceway ponds are the most pervasive commercial-scale microalgae culture systems due to ease of operation and low cost of construction and maintenance [98]. However, due to issues like contamination prevention, the need for high productivity and high-purity microalgae culture, closed microalgae culture systems or Photobioreactors have sustained their place in microalgae culture systems. The following section describes some of the essential microalgae culture systems in detail.

1.6.1 Raceway Pond system

Raceway ponds are shallow artificial water bodies used for microalgae cultivation. The ponds are usually divided into rectangular channels and oval-shaped ends, making them look like an automotive raceway circuit. The rectangular channel is fitted with a paddlewheel system to keep the microalgae culture moving around the channel continuously and prevent the microalgae from settling to the bottom. Baffles are added in the channel to maintain proper channel geometry and achieve optimum flow dynamics. Figure 1.11(a) [101] illustrates a schematic of a raceway pond system used for microalgae culture. The depth of the channels is kept at 0.15- 0.30 m for optimum light penetration [102]. Open raceway ponds are one of the most widely used commercial microalgae culture systems due to ease of construction and operation and the good economics of microalgae production. These systems date back over 60 years [103] and are still operating for commercial-scale microalgae cultivations, as shown in Figure 1.11.b culturing *Spirulina* sp. by Parry Nutraceuticals, India [104] and Figure 1.11.c culturing *Haematococcus pluvialis* by Atacama Bionatural at Atacama desert [105]. However, these systems have limitations like the need for large open lands, contamination issues, low biomass productivity, low biomass density, etc., limiting their wide-scale implementations [102].

(a) **(b)** **(c)**

Figure 1.11 (a): Schematic of a raceway pond system, **(b):** Raceway pond system by Parry Nutraceuticals, India, **(c):** Raceway pond system Atacama Bionatural at Atacama Desert.

1.6.2 Stirred circular pond system

A stirred circular pond system comprises a circular pond equipped with stirring mechanisms such as paddlewheels or rotating drums as shown in Figure 1.12. These stirring devices ensure continuous mixing of the culture medium, preventing sedimentation and ensuring uniform distribution of light and nutrients. Additionally, the pond is often enclosed with transparent materials like glass or plastic to create a controlled environment, regulating factors like temperature and CO_2 levels. The stirred circular system gained importance as a microalgae cultivation system due to its ease of operation and maintenance and low capital and operational cost [106]. Circular ponds were the first commercial-scale microalgae cultivation system used in Japan back in the 1960s to cultivate *Chlorella* microalgae species [107]. Large-scale circular systems have already reached diameters up to 50 m, as reported [108]. Still, due to a lack of scalability, contamination issues, and the need for specific geographical locations, these systems are insufficient to produce microalgae biomass needed at the quantity and quality required to substitute petroleum.


(a) (b)

Figure 1.12 (a): *Chlorella sp.* grown by Sun Chlorella USA in a circular pond system [109], **(b):** 1st commercial *Chlorella* production in Japan, 1960 [107, 110].

1.6.3 Tubular photobioreactor

Amongst the closed microalgae cultivation systems, tubular photobioreactors have gained a special place due to their capability of high biomass productivity. As shown in Figure 1.13, Tubular photobioreactors are a sleek and innovative design in microalgae cultivation, representing a controlled environment where microalgae thrive within transparent tubes. These transparent tubes allow efficient light exposure, optimizing photosynthesis for enhanced growth. The closed-system design minimizes contamination risks while facilitating easy monitoring and control over environmental factors like temperature, pH, and nutrient levels. Its modular structure offers scalability, making it adaptable to various production scales and ideal for research and commercial applications.


(a) (b) (c)

Figure 1.13 (a): A stacked tubular photobioreactor [111], **(b):** A spiral tubular photobioreactor [112], **(c):** A horizontal tubular photobioreactor [113].

However, issues like biofouling, high energy requirement for temperature control, efficient media circulation, improper gas exchange, etc., make tubular photobioreactors less viable for large-scale commercial applications.

1.6.4 Flat Plate photobioreactor

The flat-plate photobioreactor’s fundamental design comprises a transparent, flat panel where microalgae thrive within a thin layer or suspension, benefiting from ample exposure to light for efficient photosynthesis, as shown in Figure 1.14. The controlled environment allows for precisely manipulating variables like temperature, nutrient supply, and light intensity, ensuring optimal growth conditions. This design facilitates the production of diverse compounds, including biofuels, pharmaceuticals, and nutraceuticals, and offers scalability advantages. Its modular structure enables easy replication and stacking, making it an appealing choice for research and industrial applications. The flat plate photobioreactor's ability to efficiently utilize space while harnessing sunlight for sustainable production renders it a promising technology at the forefront of modern bioprocessing methodologies. Flat plate photobioreactors have advantages over other photobioreactors, such as simple design, relatively easy monitoring and maintenance, efficient light penetration, scalability, etc. However, they have certain limitations as well. While flat plate photobioreactors are scalable, scaling up may not be as straightforward as other reactor designs. Issues related to efficient gas exchange, mixing, and light distribution can become more complex as the size increases. A large surface area is needed to achieve significant production levels. This can pose challenges regarding space requirements, especially in areas with limited available land. These limitations have also affected the mass-scale adaptation of flat plate photobioreactor systems.

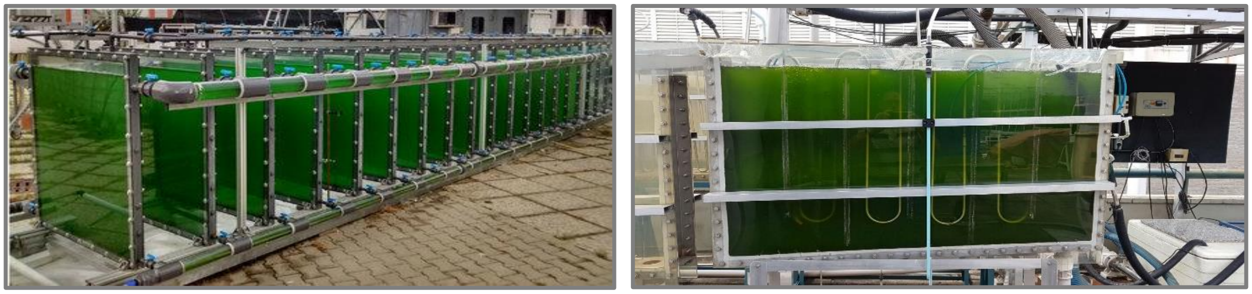

(a) (b)

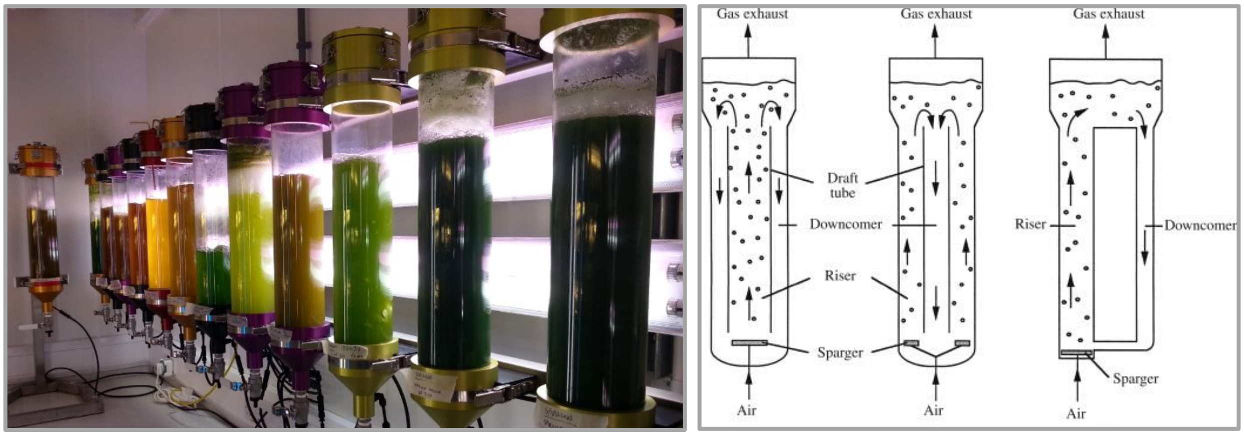
Figure 1.14 (a): A 1300 L capacity flat plate photobioreactor at CNR experimental area of Sesto Fiorentino (Firenze) [114], **(b):** An experimental flat plate photobioreactor at UFRJ, Brazil.

1.6.5 Airlift photobioreactor

An airlift photobioreactor is a type of bioreactor that uses pressurized air to circulate and mix the culture medium and the microalgae inside. Figure 1.15 illustrates airlift photobioreactors and some of their variations. Additionally, carbon dioxide from the air helps the microalgae develop and respire, as well as aids in the elimination of waste. One of the challenges of microalgal cultivation is to provide sufficient light for photosynthesis while avoiding excessive light that can damage the cells. To improve light distribution and utilization efficiency, some air-lift photobioreactors use novel designs, such as internal illumination with fiber optics [115] or inclined reflective broth circulation guides [116]. Air-lift photobioreactors have several advantages over other bioreactors, such as low shear stress, high mass transfer, simple operation, and easy scale-up.

An advantage of an air-lift photobioreactor is that it uses pressurized air to circulate and mix the culture medium and the microorganisms, while other bioreactors may use mechanical agitators, rocking motion, or fluidization. This reduces the shear stress and damage to the cells and enhances oxygen and carbon dioxide mass transfer. This also simplifies the bioreactor's design and operation, reducing the risk of contamination and fouling.

However, an air-lift photobioreactor has some challenges in scaling up, controlling the bubble size and distribution, and optimizing the light intensity and exposure.



(a) **(b)**

Figure 1.15 (a): Cultivation of different algal species in bubble columns photobioreactor at Plymouth Marine Laboratory [117], **(b):** Different airlift photobioreactor designs [118].

1.6.6 Plastic bag photobioreactor

The plastic bag photobioreactor uses a transparent plastic bag as the container for the culture medium and the microalgae, as shown in Figure 1.16. The plastic bag photobioreactor has several advantages over other types of photobioreactors, such as: (i) it is easy to set up, operate, and dispose of, as it does not require any complex components or structures, (ii) it is flexible and adaptable, as it can be shaped and arranged according to the available space and light conditions, (iii) it is low-cost, as it uses inexpensive and readily available materials, such as plastic film and air pumps.

There are different designs of plastic bag photobioreactors, depending on the bag's shape, size, orientation, and configuration. Some examples are:

(a) Hanging bag type: This is a simple and low-cost photobioreactor that consists of a plastic bag hung from a support, such as a frame or a pole, as shown in Figure 1.16 (a). The bag is operated as a bubble column, where pressurized air is injected at the bottom to circulate and mix the culture medium and the microalgae. In addition to facilitating waste product removal, the air delivers oxygen and carbon dioxide needed for the microalgae's development and respiration. The hanging bag photobioreactor can be used for indoor and outdoor cultivation, and can be easily scaled up by increasing the number and size of the bags [119, 120].

(b) Horizontal bag type: This novel and innovative PBR consists of a thin film plastic bag laid horizontally on a flat surface, such as a roof or a ground, as shown in Figure 1.16 (b). The bag can also be operated as a rocking motion bioreactor, where the bag is periodically tilted from one side to another by a mechanical device, such as a motor or a windmill. The rocking motion creates a wave-like movement inside the bag, which enhances the mixing and mass transfer of the culture medium and the microalgae. The horizontal bag PBR can also use solar energy to power the rocking device and achieve high biomass productivity and photosynthetic efficiency [121, 122].

(c) Vertical panel bag type: This sophisticated and advanced photobioreactor consists of a plastic bag arranged in multiple vertical panels, as shown in Figure 1.16 (c). The bag is operated as a flat panel reactor, where the culture medium and the microalgae flow continuously through the panels, illuminated by natural or artificial light sources. The vertical panel bag PBR can optimize light

distribution and utilization efficiency and achieve high biomass concentration at a low investment cost.

Plastic bag PBRs are promising systems for microalgal cultivation, as they can overcome some of the limitations of conventional PBRs, such as high cost, low scalability, and low sustainability. Plastic bag PBRs can also produce various microalgal species and products, depending on the microalgae's cultivation conditions and genetic engineering. However, the cost of replacing the plastic bags due to wear and tear, biofouling, etc., adds up to the overall cost of production of the microalgae biomass.

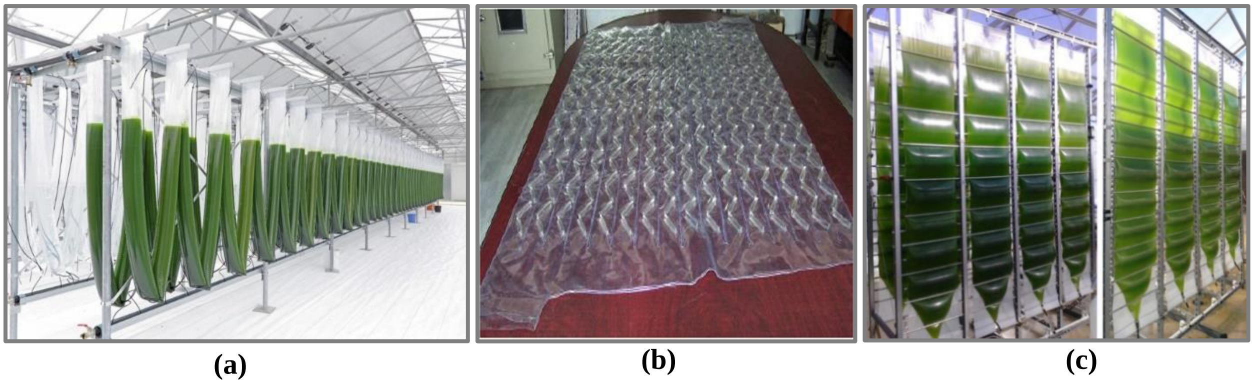
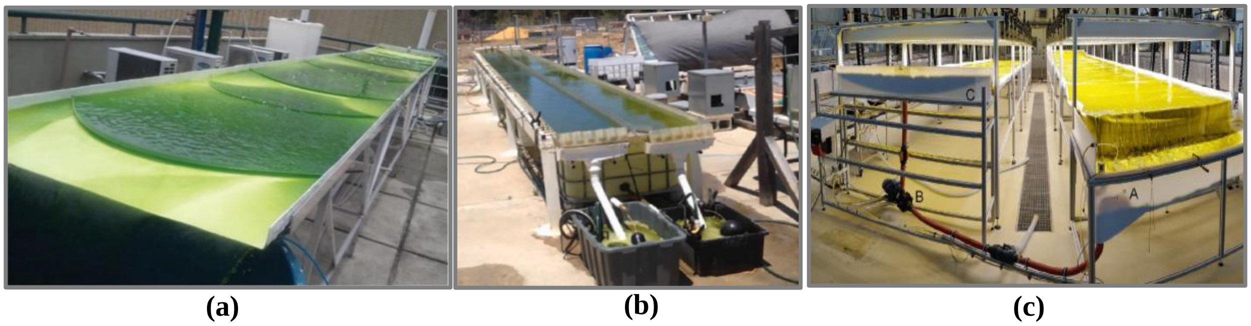

(a) (b) (c)

Figure 1.16 (a): Hanging bag type photobioreactor [123], **(b):** A horizontal thin film bag photobioreactor [124], **(c):** A vertical panel type bag photobioreactor [125].

1.6.7 Descending film

Descending film type microalgae cultivation is a method of growing microalgae on a thin layer of liquid medium that flows down an inclined surface, as shown in Figure 1.17. Compared to traditional suspended growing techniques, this approach offers a number of benefits, including a greater biomass production, less energy and water usage, and simpler harvesting. In 1970s, Czech Republic developed these systems [126]. Typically, the film size in these systems is about 1cm thick and supports cell concentration of up to 10 g L^{-1} [126]. The systems were initially more expensive because of their glass bases, but as they have evolved and modern, less expensive materials like plastic, cement, metal, etc. have been used, the cost of the systems has decreased. These systems have a few benefits over other closed reactors, including a lower cost due to their thin culture layer, effective gas and liquid transmission, and a high cell concentration. Similar to raceway ponds, descending film systems have a high potential for contamination even though they have good production[126].



(a) (b) (c)

Figure 1.17 (a): A descending thin-film photobioreactor at GreenTec laboratory, UFRJ, Brazil [126], **(b):** A cascading type thin film descending photobioreactor [127], **(c):** An artificially illuminated thin film descending type photobioreactor [128].

1.6.8. Internally illuminated photobioreactor

Researchers are exploring internally illuminated microalgae culture systems to achieve proper light distribution using technologies like plastic optical fiber cables (POFC), as shown in Figure 1.18 (a). As part of a Department of Biotechnology (DBT), Government of India sponsored Indo-Brazil collaborative project (DBT/IC-2/Indo-Brazil/2016-19/04), I was fortunate enough to visit Brazil and study the photobioreactor in picture, which is a 1000L capacity internally illuminated photobioreactor developed in the Green Tech laboratory, Federal University of Rio de Janeiro (UFRJ), Brazil. Fresnel lens setup with an Infrared (IR) filter, as shown in Figure 1.18 (b), captures and concentrates sunlight at a point. The concentrated sunlight is then transferred to the microalgae culture through POFCs and distributed inside the microalgae culture by arranging the POFCs in an optimized pattern, as shown in Figure 1.18 (c). Air mixed with carbon dioxide is blown from the bottom using metal spargers to keep the microalgae floating and provide the necessary carbon to the microalgae culture. The flow of carbon dioxide is controlled by monitoring the pH of the culture using a pH probe. This type of system has the advantage of achieving high growth rates and low operational costs due to free sunlight. However, the cost of the system is too high as many POFCs are needed to collect and transport the sunlight from the point of collection to the point of utilization. Additionally, problems like proper distribution of the sunlight at the microalgae culture were also an issue as the POFCs emitted the light from its end, which has a minimal cross-sectional area, and thus, the sides of the POFCs needed to be itched or scraped to increase the area for the light to exit from the POFCs. Another issue with such a system was biofouling, which led to microalgae growth on the POFCs and blocked light. It also required frequent cleaning, which is a tedious, labor- and energy-intensive process. Thus, systems will not be sustainable at an industrial scale operation, these types.


(a) (b) (c)

Figure 1.18 (a): A 1000 L capacity POFC-based internally illuminated microalgae culture system at Greentec laboratory UFRJ, Brazil, **(b):** A Fresnel lens and infrared filter assembly collecting sunlight and transferring it to POFC, **(c):** POFC and air sparger assembly of the internally illuminated photobioreactor system

1.7 Problem statement and objectives of the study

As previously stated, over 90% of marketed microalgae are still generated in open ponds [98]. Open system is economical but have limitations like contamination issues, low biomass productivity, difficulty for mano culture of microalgae species, etc. Most importantly, open systems require large open areas with favorable environmental conditions, making widescale implantation of open systems difficult.

To fill the vast gap of the open culture system, closed microalgae culture systems are being developed. In addition to the microalgae culture systems mentioned in section 1.6, many more innovative microalgae culture systems are being developed to date. However, due to various limitations like high capital and operational costs, issues of inefficient mass transfer, biofouling, etc., the prevailing closed microalgae culture systems are not able to be commercially successful. The current study is carried out to address these issues with the following objectives.

Objectives:

1. To design and develop a photobioreactor (PBR) for mass-scale microalgae cultivation.
2. To optimize the culture conditions and improve the biomass and lipid productivity.
3. To analyze the algal biomass and biofuel properties produced from the microalgae cultured in the developed PBR.

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