

# Nature-based Solutions for the Management of Environmental Pollution and Resource Recovery



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Edited by

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and Shubha Dwivedi

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## PREFACE

As the world faces unprecedented environmental challenges, the need for innovative and sustainable solutions has to be requisite to overcome these challenges. The burdens of modern society have led to an increase in environmental pollution and resource depletion, posing severe threats to both human health and ecosystems. Traditional methods of pollution control and resource management, while effective to an extent, often involve high costs, energy consumption, and unintended environmental consequences.

In response to these challenges, Nature-Based Solutions (NBS) have emerged as a promising approach to address environmental pollution and resource recovery. NBS are inspired by and leverage the natural processes and ecosystems to mitigate environmental issues, offering a sustainable and cost-effective alternative to conventional methods. These solutions range from the restoration of wetlands to improve water quality, carbon sequestration, green infrastructure in urban areas to manage stormwater, phytoremediation of contaminated soils using plants. The resource recovery process is to extract valuable materials or energy from waste, is another area where NBS can play a transformative role.

The integration of NBS into environmental management represents a shift towards a more holistic approach that not only addresses the symptoms of pollution but also enhances the resilience of ecosystems and supports biodiversity. Moreover, NBS often provide co-benefits such as carbon sequestration, habitat creation, and enhanced aesthetic and recreational opportunities, contributing to the overall well-being of communities.

As the global community seeks to meet the Sustainable Development Goals (SDGs), particularly those related to clean water, clean energy, and sustainable cities, NBS offer a pathway to achieving these objectives in a way that is both effective and sustainable.

This book aims to explore the various aspects of NBS in the management of environmental pollution and resource recovery. It will provide insights into the principles, applications, and benefits of NBS, as well as case

studies demonstrating their effectiveness in different contexts. By showcasing the potential of NBS, we hope to inspire researchers, policymakers, and practitioners to adopt and further develop these solutions, ultimately contributing to a more sustainable and resilient future for our planet.

As we delve into the content, it is crucial to recognize that while NBS offer immense potential; their implementation requires careful planning, interdisciplinary collaboration, and ongoing monitoring to ensure their success. The chapters that follow will discuss these challenges and provide guidance on how to overcome them, making NBS a viable and mainstream option in environmental management.

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# CHAPTER 1

## SUSTAINABLE BIOENGINEERING SYSTEMS FOR FIXING ENVIRONMENTAL POLLUTIONS

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### Abstract

Environmental pollution caused by human activities poses significant challenges to the health and well-being of our planet. Traditional approaches to remediate pollution often involve costly and resource-intensive methods, which may not be sustainable in the long run. In recent years, bioengineering has emerged as a promising field that offers sustainable solutions to address environmental pollution. Bioengineering involves the application of biological principles and processes to design and implement innovative technologies that harness the power of nature to restore and preserve the environment. Bioengineering systems utilize natural sources such as different organisms including bacteria, fungi, plants, algae and other biomaterials, to degrade organic contaminants, absorb toxins or converted to less toxic forms, and restore ecosystems. This chapter focuses on a comprehensive overview of sustainable bioengineering systems and their applications in pollution remediation and recovery of valuable products by emphasizing the importance of sustainable practices in bioengineering. This chapter also highlights the significance of bioengineering techniques in harnessing the power of nature and integrating it with sustainable practices to fix the intricate environmental issues. Bioengineering offers innovative and effective

solutions to restore our ecosystems in a sustainable way and ultimately provide a safe and healthy future for our next generations and also help us to achieve the United Nation's Sustainable Development Goals (SDGs).

**Keywords:** Bioengineering, sustainable development, environmental pollution, biochar, natural organisms

## 1. Introduction

Earth is an astonishing place in the universe that boasts unique and unparalleled life forms. From the grandeur of the blue whale to the tiniest of microorganisms, life on Earth is a wonder to behold. Every species, large and small alike, contribute to the planet's vibrant biodiversity. It is becoming increasingly inhospitable to most species, as indicated by the steep decline in flora and fauna diversity. All of these factors have caused a drastic decrease in the quality of life on Earth. Anthropogenic activities are primarily to blame for the planet's deteriorating environmental circumstances. Increased greenhouse gas concentration and their contribution to global warming as shown in Figure 1, land degradation, loss of biodiversity, pollution of the air, water, and soil, climate change, exhaustion of resources that are not renewable, accumulation of hazardous recalcitrant compounds, and several other related problems are all the result of human meddling. This has had a profound impact on human health, livelihoods, and economies around the world. Solutions are necessary to mitigate the effects of anthropogenic activities and protect the planet (Arora et al., 2008). According to Vezzoli and Manzini (2008), environmental sustainability is a concept that seeks to ensure a balance between human activities and the environment. It involves preserving natural capital and establishing systemic conditions that prevent human actions from interfering with natural cycles more than natural resilience allows. This system also ensures that natural resources are not depleted and can be shared with future generations. Human activity is primarily responsible for the growing environmental issues around the world. Land availability, environmental quality, and biodiversity have all steadily declined over the past century, and by 2050, even worse conditions are predicted. To meet environmental sustainability goals, biological techniques must take the lead and be used as much as possible. Currently, the globe is experiencing a number of significant environmental issues that have a grave influence on biological forms. The section below discusses the environmental issues that stand out the most (Arora et al., 2008).

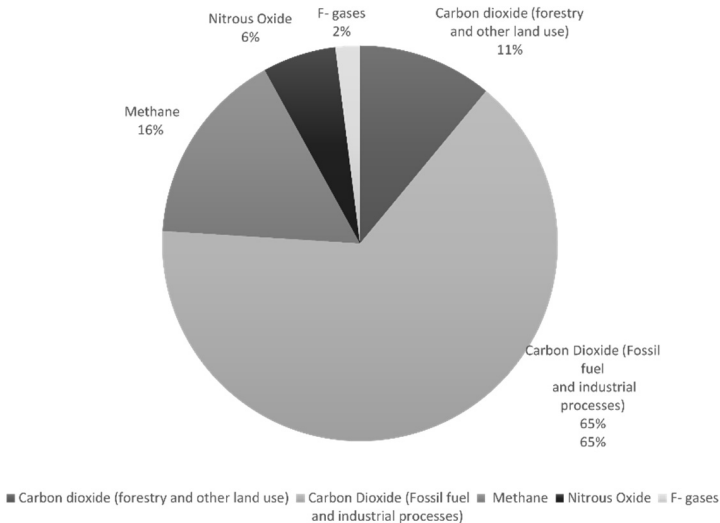


Figure 1: Contribution of Greenhouse gases to global warming

## 2. Background and Impacts of Environmental Pollution

When dangerous compounds or excessive levels of certain natural elements contaminate the environment, this is referred to as environmental pollution. Over the past few centuries, it has become a more significant and complex worldwide issue. There are many other ways that pollution can manifest itself, including through the air, water, soil, noise, and light. Human activities like industrialization, transportation, agriculture, and inappropriate waste disposal are its main causes. Environmental pollution affects every part of the world. Pollution affects all types of life in some way. In places that people do not even inhabit, such as the poles and deep underwater, pollution can have an impact on organisms. In recent decades, numerous contaminants have emerged due to human activity, negatively affecting ecosystems (Rockström et al., 2009). Conditions in developing nations are worse because the ecosystems are being damaged by rife industrialization, rapid deforestation, and urbanization. Landrigan et al. (2017) reported that emerging nations account for about 92% of fatalities related to pollution. Sole-Ribalta et al. (2016) contend that urban areas have become congestion hotspots that threaten air, water, and soil quality. A UN assessment predicts that the population of cities will double by 2030 (Arora et al., 2008). The cost of pollution-related financial losses is

estimated to be US\$ 4.6 trillion annually or 6.2% of global economic output. Healthcare expenses are rising as a result of environmental risk factors, particularly pollution in the air and non-communicable illnesses, and are expected to reach \$8.1 trillion in 2019, or 6.1% of world GDP as per the World Bank pollution report. According to Landrigan et al. (2017), different types of pollution in low- and high-income nations can push up the yearly healthcare budget by 1.7% and 7%, respectively. Although the exact costs of cleaning up contaminated soil, water, and air have yet to be calculated. Every year, industrial emissions release a variety of contaminants into the environment. According to data from Landrigan et al. (2017), more than 1,40,000 new chemicals and pesticides have been developed since 1950, but only a small subset of these chemicals, like polychlorinated biphenyls, polyethylene, dichlorodiphenyltrichloroethane, hydrochlorofluorocarbons, and chlorofluorocarbons, have gone through extensive toxicological testing. It is commonly known that CFCs contribute to the thinning of the ozone layer. Thankfully, the Montreal Protocol eliminated CFCs that were harming the ozone layer (NASA 2018). But in recent years, ozone depletion research has shown that the level of the lower stratosphere over non-polar regions is diminishing (Arora et al., 2008). In non-polar locations, ozone depletion is thought to be caused by chemicals used in the paint industry. The consumption of fossil fuels to meet the world's enormous energy demand is one of the main causes of air pollution and global warming, which are covered in the sections that follow.

Another xenobiotic and recalcitrant pollution created by humans that is wreaking havoc on the planet is plastic. Due to the harm, they do to ecosystems all around the world, plastics are an ecological and environmental disaster. Only the Pacific Ocean receives roughly 79,000 tonnes of plastic waste annually. The Pacific Ocean's "great Pacific garbage patch" is three times the area of France (Dailymail.co.uk 2018). 73% of mesopelagic fish caught in the Northwest Atlantic Ocean had microplastics in their intestines, according to an investigation conducted by Wicczorek et al. (2018). There is a considerable chance that microplastics will make their way through the food chain in the surroundings because many marine animals consume mesopelagic fish. The production of these hazardous materials is steadily increasing at a rapid rate despite the horrible impacts of plastics, and the globe has become a landfill for these unbreakable objects. Oil spills are another significant anthropogenic activity that has a negative impact on the world's marine ecosystems. Every year, millions of barrels of oil are spilled into the oceans as a result of incidents involving pipelines, tankers, or offshore

oil rigs (Arora et al., 2008). Oil spills over the oceans reduce the amount of oxygen available, block the sun's light from reaching the water, and cause poisonous components to build up and disperse, killing off beneficial bacteria and even causing the extinction of keystone species. According to Liu and Kujawinski (2015), oil spills have short- and long-term destructive effects on the environment at local, regional, and global scales. Polar and non-polar, volatile, and non-volatile components of spilled oil have different effects on flora, fauna, and the ecosystem as a whole. Due to physical impacts like coating, smothering, and persistence, oil spills have also been linked to some mechanical harm to organisms and environments.

Heavy metals and other toxins have been damaging land and water as a result of rapid urbanization, industrialization, and related anthropogenic activities (Arora et al., 2008). Even while the addition of heavy metals is a normal process, human activity is raising their concentration to unnatural levels (Mishra et al., 2017). Industrial and agricultural wastewater, nuclear power plants, domestic waste, metallic rust, petroleum hydrocarbon spills, metal polishing, mining, industrial processes like refineries, plastics, burning fossil fuels, textiles, paints containing lead, smelting, waste from electronics, pharmaceutical substances, agrochemicals, incineration of garbage, and vehicle exhausts are all known sources of heavy metals in soil. Around the world, ecosystems of soil and water have been found to be contaminated with heavy metals. Due to lead poisoning in the lake, Flint (Michigan), in North America, switched its water source from Lake Huron to the Flint River in 2014. Due to serious lead pollution of drinking water, the issue further poisoned the water source, and the US President declared an emergency in January 2016. Over 42 Indian rivers were recently discovered to be polluted with dangerous heavy metals, based on a report on (weather.com 2018). In addition to this, a new investigation indicates that even Mount Everest has been contaminated with heavy metals, with samples revealing the presence of As and Cd at levels beyond the US Environmental Protection Act's (USEPA) permissible limits.

## **2.1 Global Warming and climate change**

Numerous elements, including the sun's energy output, volcanic eruptions, the concentration of GHGs in the atmosphere, and aerosols, influence the earth's temperature. Without a doubt, the global climate has been changing since the beginning of the industrial revolution. The pace of climate change is accelerating due to an increase in human-caused events such as industrialization, urbanization, deforestation, contemporary agricultural

practices, changes in land use patterns, and many others (Arora et al., 2008). The phrase "climate change" often refers to changes over time in factors such as precipitation, wind, and temperature, according to Parry et al. (2007). Since the middle of the 20th century, human activity has been the dominant cause of global warming, pursuant to the United Nations Panel on Climate Change. Climate change and the pace of carbon dioxide emissions are strongly correlated. According to the IPCC (2013), rising emissions and accumulation of GHGs such as carbon dioxide, methane, nitrous oxide, water vapor, hydrofluorocarbons, perfluorocarbons, and sulphur hexafluoride are the main causes of climate change at the moment. According to estimates, atmospheric GHG concentration has increased. For example, CO<sub>2</sub> concentration increased to 465 ppm in 2020, which is about 49 ppm more than it was ten years ago and about 185 ppm more than it was during pre-industrial times. CH<sub>4</sub> concentration has increased from 715 to 1874 ppb from 1750 to 2012 (IPCC (2013), and the level of N<sub>2</sub>O rose from 227 to 323 ppb. According to reports, GHGs have reached their highest levels in Earth's history and are still rising. Even if emissions stopped immediately, the climate would still shift for years to come. The IPCC predicts that, with large regional differences, global surface temperatures would rise by 1.4-5.8 °C by 2100, up 0.74 °C since the late 19th century. It is commonly recognized that CO<sub>2</sub>, which currently accounts for 76% of the whole impact of global warming, is the most significant GHG. Most nations in the world are producing more CO<sub>2</sub>, along with some nations that contribute significantly (%) to global CO<sub>2</sub> emissions as shown in Figure2. Bhutan is the sole nation with a negative carbon value, despite the fact that the world's CO<sub>2</sub> level is rising, as a result of its region having the highest percentage of forested land (Arora et al., 2008). The primary cause of carbon emissions is the use of fossil fuels. According to estimates, the consumption of fossil fuels made up 84% of the world's primary energy supply in 2015. According to Loehman (2010), the effects of climate change include unusually warm weather, polar warming, melting glaciers, heavy precipitation events, coral reef bleaching, prolonged droughts, changes in plant and animal distribution, and an increase in environmental degradation and natural disasters. According to data from Vaughan et al. (2013), the Arctic's ice has consistently decreased from 1979 to 2020 by 2.6% in the winter (March) and 13.1% in the summer (September). In the past century, the average sea level rose by 12–22 cm, and it is expected to rise by another 24–30 cm by 2065 and 40–63 cm by 2100, which will cause a reduction in land area (Arora et al., 2008). Ocean thermal expansion brought on by global warming has worsened the situation and reduced the amount of land that is

available. By 2050 and 2080, respectively, the size of the permafrost region will have decreased by around 20–35% and 30–50%, respectively, as a result of global warming, releasing harmful pathogenic bacteria and viruses that were previously buried in dormant form.

Direct and indirect effects of climate change on soil microbe-microbe and plant-microbe interactions, as well as a decrease in soil microbial diversity, have all been documented (Arora et al., 2008). In addition, dissolved organic matter release and pathogen occurrence are speeding up due to climate change, increasing the potential for contagious illnesses in people, wildlife, and even plant life (Williamson et al., 2017). The infections, vectors, hosts, and ecosystems of infectious diseases that afflict humans are all impacted by climate change. Major diseases' spread and intensity are impacted by climate change and extreme weather. It is projected that the rate of soil organic matter breakdown would accelerate with rising global temperatures, which will lead to a significant CO<sub>2</sub> release and a subsequent loss of soil fertility. The details have been covered in the next section, however, changes in climatic conditions have an impact on agricultural output and food security both directly and indirectly.

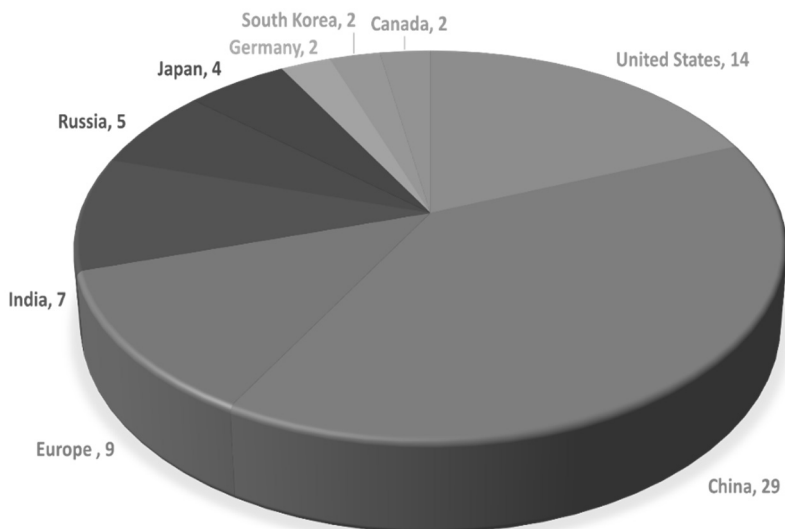


Figure 2: Major Carbon dioxide producing Countries (CO<sub>2</sub> level in Million Tonnes)

## ***2.2 Restrictions on Agriculture and land deterioration***

Land degradation is a problem that affects all life forms and is not just limited to the deterioration of soil quality. The deterioration of the entire ecosystem, including the biological cycles, related biodiversity, and ecosystem services such as carbon sequestration, is also connected to it. The UN Convention to Combat Desertification has classified land degradation as a global problem with worrying effects on the productivity and health of soil resources (Arora et al., 2008). According to the Global Soil Partnership (headed by FAO), the annual cost of degrading arable land soil is estimated to be \$75 billion tonnes (Petagram), or \$400 billion USD (FAO (2017). According to Arora (2018), land degradation has a direct impact on the lives of 1.5 billion people around the world and results in the loss of 15 billion tonnes of fertile soil each year. Agro-ecosystems are also vulnerable because croplands alone account for 20% of all degraded lands worldwide, which results in an estimated income loss of US\$10.8 billion ([un.org/en/events/desertification](http://un.org/en/events/desertification)). According to FAO (2017), climatic variability has a substantial role in land degradation and affects agricultural regions' fertility and output.

According to FAO (2017), major agricultural crops have seen considerable production declines worldwide as a result of the warmer temperature, especially the Rabi crops like wheat. According to the IPCC, grain yields decrease by around 5% for every degree that the temperature rises (Arora et al., 2008). The current heat wave and unusual temperature spike in Europe have wreaked havoc on crops and resulted in the poorest harvests since the conclusion of World War II. Formerly significant exporters of food, such as Hungary, Bulgaria, and Romania in Europe, are now importing it. The former Soviet Union's breadbasket, Ukraine, saw a 75% decline in wheat production in 2018 compared to the previous year on average. Unusual precipitation patterns and rising global temperatures are also to blame for the frequent occurrence of severe weather events like drought and floods, which exacerbate wind or soil erosion (FAO 2017).

In addition to raising the temperature to over 40 °C, the latest heat wave in Europe is also triggering drought and wildfires around the continent. Desertification is a worrying side effect of the worrisome groundwater loss scenario that is developing in India. In comparison to other parts of the world, Northern India has the largest groundwater declination, according to a NASA assessment. According to NASA, groundwater levels in Haryana, Rajasthan, Punjab, and Delhi dropped by 108 cubic kilometers between 2002 and 2008. Future effects include a severe water shortage



that will cause drought in these regions ([wri.org/blog/2015](http://wri.org/blog/2015)), and as of June 25, 2019, nearly 65% of the nation's reservoirs were dry. Six of the states of Maharashtra's 17 reservoirs have dried up, making it one of the worst-affected regions (Arora et al., 2008). Owing to escalating withdrawals and other human impacts, even the largest lakes and wetlands are under stress from the drought. For instance, the second-biggest saltwater lake, Lake Urmia, has decreased in size by 80% over the past 40 years, with a considerable decline happening between 2009 and 2015. This decline is mostly attributable to the construction of some 20 artificial dams around the lake to redirect the flow of water to agricultural fields. Due to salty storms from the hypersaline lakebeds brought on by this significant desiccation, the surrounding land has degraded. With an estimated area of 1 billion hectares, soil salinization is another significant factor in land degradation that affects most countries. According to Arora (2018), there is a connection between drought and land salinization. The main contributors to land salinization include agricultural practices and the use of ineffective irrigation techniques. Low-quality irrigation water causes salt to build up in the soil, and inadequate drainage further makes the situation worse. The stability of pesticides like parathion increases in saline soils, making them infertile. Agriculture fields have been impacted by salinization, which has an impact on plant yield and food security. Approximately 10 million hectares of agricultural land are negatively impacted by salt each year (Arora et al., 2008). One-third of the world's food is produced on irrigated lands, 20% of which are affected by salinity. Crop growth and output are severely hampered by salinity, especially in vegetable crops, which have a very low tolerance for salt stress. Poor moisture retention capacity, elevated electrical conductivity (4 dS/m or more), nutritional imbalance, and increased susceptibility to hunger, drought, and soil erosion are all characteristics of saline soils (Arora et al., 2008). Reactive oxygen species are produced in plants as a result of salinity, which also causes Na<sup>+</sup>, Cl toxicity, ethylene stress, plasmolysis, osmotic imbalance, nutritional insufficiency, reduced photosynthetic rate, partial stomatal closure, and nutrient deficit. Additionally, salt toxicity impairs the diversity, function, abundance, and qualities of advantageous soil bacteria. By 2050, more than 50% of agricultural land will be saline if the current trend continues (Arora et al., 2008).

### ***2.3 Loss of Habitat and Biodiversity***

The loss of biological variety has developed into a challenging and continuous problem. This has therefore resulted in an unprecedented

reduction in land and aquatic life including flora and animals, jeopardizing the ecosystems' general stability. This has reduced biological heterogeneity. According to reports from the World-Wide Fund for Nature (2014), 52% of the world's biodiversity disappeared between 1970 and 2010. The biggest issue is the disappearance of plant species since they are crucial for keeping the ecosystem in balance and have a direct impact on how it functions by providing a habitat for a variety of other animals. Since the past 40,000 years, human-caused extinctions have been more severe. According to predictions made by Barnosky et al. (2011), the world will likely experience its sixth mass extinction within 240 years if current trends are kept up. In fact, some experts contend that humans alone are to blame for the sixth major extinction of species, which is currently under progress. In 43 percent of the globe's terrestrial surface, it is estimated that there have been disturbances and natural vegetation has been replaced by artificial ecosystems. In this century, extinction rates are predicted to climb by a factor of two, and as climate change intensifies, this process could accelerate even more. The main challenges to biodiversity are habitat loss and fragmentation, both of which depend on one another. Natural habitats have undergone a metamorphosis that has resulted in their degradation and has posed serious risks to the natural environments of plants and animals. Due to the population boom, there is an increased demand for resources, which has led to an increase in infrastructure construction, mining operations, and land usage for agriculture. Oceans encompass over 72% of the earth's surface, making up more than 95% of the entire biosphere. Organization for Economic and Cooperative Development (OECD) (2016) estimates that 16% of all animal protein consumed globally, or almost 2.6 billion people, is derived from oceans. UNDESA (United Nations Department of Economic and Social Affairs 2017) cites mangroves and other vegetated ocean ecosystems as "Blue Carbon" sinks because they capture 25% of the CO<sub>2</sub> produced by fossil fuels and protect coastal communities from storms and swells. However, anthropogenic activities have had an influence on the oceans all over the world (Arora et al., 2008). This has reduced oceans' ability to supply ecosystem services, and the environment's ability to fend off human activity is now in jeopardy. In actuality, the planet's oceans and poles today have some of its most vulnerable ecosystems. The environment is changing dramatically and randomly, which could have negative effects on ecosystems all around the world. According to current estimates, global warming is to blame for 34% of the overall loss of biodiversity. As was said in the preceding section, climate change and global warming alter the geographic distribution of species and raise the likelihood that some may

become extinct, especially in habitats that are shrinking as a result of changes in the climate (Parry et al., 2007). If estimates are to be accepted, 15–37% of terrestrial species are predicted to go extinct by 2050 due to global warming. According to several meta-analysis studies (Forrest et al. 2010), the life events of species are changing as a result of warming temperatures. Rising temperatures and the frequency of heat waves (both on land and in the oceans) are putting marine species at even greater risk. This is due to the fact that marine life is less adapted to temperature changes than creatures on land (Arora et al., 2008).

### **3. Bioengineering Principles and Approaches for Sustainable Practises**

Sustainable bioengineering, commonly referred to as biological engineering, is a cutting-edge and multidisciplinary field that blends biological, engineering, and environmental science principles to create sustainable solutions for the myriad problems facing our world. It entails using biological systems as a platform for the application of engineering concepts and methods for a variety of goals, including the removal of pollution. It makes use of living things like plants, microbes, and enzymes to break down, change, or remove environmental toxins. A variety of promising solutions that can lessen environmental pollution and advance sustainable development are offered by the discipline of sustainable bioengineering. Sustainable bioengineering seeks to maximize the positive effects of human activity on the environment while simultaneously producing useful goods and services by utilizing the power of biological systems. Bioengineering and its potential for pollution remediation are shown in Figure 3.



## **4. Cleaning Up Environmental Pollutants with Bioengineering Methods**

Due to its promise to deliver long-lasting and ecologically beneficial solutions, bioengineering techniques for pollution clean-up have attracted a lot of attention. These methods employ biological systems including plants, microbes, microalgae, nanoparticles and enzymes to break down, change, or immobilize contaminants in soil, water, or the atmosphere. The following are some typical bioengineering methods for cleaning up pollution:

### ***4.1 Microalgae Bioengineering***

Environment, interest in, and output of biofuels have all rapidly increased. The largest contributor to greenhouse gas emissions and rising atmospheric CO<sub>2</sub> concentration presents significant obstacles for global sustainability and pro-environment movements. Physicochemical adsorption, injection into deep oceans or geological formations, and improved biological fixation are currently accessible technology for CO<sub>2</sub> capture. The physicochemical adsorption process is challenging to regulate, and the adsorbent materials are frequently pricy and non-renewable. Abiotic approaches, such as mineral carbonation of CO<sub>2</sub>, present substantial hurdles due to large space requirements and probable leakage over time. These methods include direct injection of CO<sub>2</sub> into the deep ocean, geological layers, old coal mines, oil wells, or saline aquifers. Energy production from low-carbon-emission sources is one of the most environmentally responsible strategies to reduce greenhouse gas emissions related to energy production. Biofuel is viewed as an efficient and feasible alternative transportation fuel that may, in the future, significantly contribute to the reduction of CO<sub>2</sub> emissions related to transportation as research and development into new energy sources advances (Zeng et al., 2011). Microalgae have a variety of benefits over other plant feedstocks in terms of CO<sub>2</sub> capture and the production of bio-oil (Srinivasan et al., 2020). These consist of (i) high rates of photosynthetic conversion, (ii) high rates of biomass production, (iii) the ability to create a variety of feedstocks for biofuels, (iv) the capacity to flourish in a variety of ecosystems, (v) made a distinction between environmental bioremediation techniques like water purification and CO<sub>2</sub> fixation from flue gas or the atmosphere (Zeng et al., 2011). vi) a lack of ability to compete for land planted with crops, and vii) a lack of ability to compete in the food market. Unicellular microalgae, unlike plants, do not divide substantial amounts of biomass into supporting

structures like stems and roots, which are energy-intensive to develop and frequently challenging to harvest and refine for use in the generation of biofuel. Additionally, photorespiration is prevented by the carbon-concentrating processes seen in microalgae. The following procedures are included in the microalgal biodiesel production system: growing, harvesting, dewatering, extraction, and trans-esterification (Radakovits et al., 2010).

#### **4.1.1 Enhancement of biofuel production**

It is possible to improve the enhancement of biofuel production in microalgae by genetic engineering. Utilizing genetic modification to increase the expression of storage proteins, lipids and carbohydrates in microalgae can increase the yield of biofuel production. Additionally, engineering the metabolism of microalgae to increase the rate of carbon dioxide uptake can also improve biofuel production. Finally, introducing genes that enhance the stability of the microalgae can help to increase the efficiency of biofuel production. Here are some strategies used in microalgae bioengineering for enhancing biofuel production:

##### **4.1.1.1 Over expression of key enzymes**

Microalgae can create more carbohydrates and proteins by over expressing the enzymes needed for their production. ADP-glucose pyrophosphorylase and starch synthase, two important carbohydrates stored by microalgae, are examples of genes whose expression can be increased. The primary biochemical in all plants, including microalgae, that stores energy is called starch. The rate-limiting molecules for starch synthesis are 3-phosphoglyceric acid (3-PGA) and adenosine diphosphate-glucose pyrophosphorylase (AGPase) (Radakovits et al., 2010). There has been a lot written about how agricultural plants might use AGPases' catalytic and allosteric characteristics to produce more starch. Some AGPases, such as Mos (1-198)/SH2 AGPase, have activity even in the absence of an activator. However, the majority of cellular AGPases are far from the pyrenoid, which may lead to 3-PGA deactivation. Starch synthesis is enhanced in several microalgal species when Mos (1-198)/SH2 AGPase or other AGPases are over expressed (Zeng et al., 2011).

##### **4.1.1.2 Manipulating regulatory genes**

Targeting regulatory genes that manage the expression of genes involved in protein and carbohydrate metabolism is possible through genetic engineering. By altering these regulatory genes, one can affect the balance

of the metabolism as a whole and allocate more energy to protein and carbohydrate storage (Zeng et al., 2011). Here's an overview of how this can be achieved:

- *Transcription Factors*: Proteins called transcription factors bind to certain DNA sequences to control the expression of target genes. By introducing genes encoding transcription factors into microalgae, their regulatory activity can be modified. As a result, the expression of genes related to protein and glucose metabolism can be precisely controlled. To increase the expression of particular genes linked to protein folding, glucose synthesis, or other important metabolic pathways, transcription factors can be engineered.
- *Promoter Engineering*: DNA regions known as promoters control the start of gene transcription. The expression levels and patterns of target genes involved in protein and carbohydrate metabolism can be modified for biofuel production by altering their promoter regions. Synthetic promoters with increased activity or those that react to particular environmental stimuli can be created and introduced using genetic engineering techniques. Promoter editing can make target genes engaged in desired metabolic pathways more effectively transcribed.
- *RNA Interference (RNAi)*: In microalgae, the expression of regulatory genes can be reduced by RNA interference. RNAi can cause the target mRNA to degrade by introducing tiny interfering RNAs (siRNAs) that precisely target and bind to the mRNA of regulatory genes, resulting in decreased production of the regulatory gene. With this method, the generation of biofuel can be modified by precisely controlling the activity of particular regulatory genes involved in protein and carbohydrate metabolism.

#### 4.1.1.3 Introduction of novel genes

Genes from other organisms can be inserted into microalgae through genetic engineering to enhance their ability to store proteins and carbohydrates. To enhance the folding and stability of carbohydrates or proteins in microalgae, the introduction of genes encoding chaperones or carbohydrate-binding modules (CBMs) can be a valuable strategy. These genetic modifications can boost the stability of carbohydrate structures, increase the effectiveness of protein folding, and reduce protein misfolding and aggregation. A brief overview of various methods is given below:

- *Chaperones*: Proteins called chaperones help other proteins fold properly and stop them from misfolding or aggregating. The cellular apparatus responsible for protein folding can be strengthened by adding genes encoding chaperones into microalgae, such as heat shock proteins (HSPs) or chaperonins. Proteins are supported by chaperones in order to ensure appropriate folding and stability and maintain their functional integrity. When microalgae are exposed to stressful situations or when expressing heterologous proteins that are prone to misfolding, this method can be especially helpful (Barati et al., 2021).
- *Carbohydrate-Binding Modules (CBMs)*: CBMs are protein domains that are not catalytic but have the ability to bind selectively to carbohydrates (Ji et al., 2020). It is possible to increase the affinity and stability of carbohydrate structures by introducing genes encoding CBMs into microalgae. CBMs can help complicated carbohydrate molecules like cellulose and hemicellulose fold and assemble correctly. They may also improve the availability of carbohydrate substrates to the enzymes needed for either biofuel production or the metabolism of carbohydrates. Microalgae may increase the efficiency of biofuel production by utilizing carbon sources and optimizing the interaction between CBMs and carbohydrates.

#### 4.1.1.4 Optimization of carbon and nutrient uptake

Optimizing carbon and nutrient uptake in microalgae is crucial for enhancing biofuel production. Efficient uptake and utilization of carbon dioxide (CO<sub>2</sub>) and nutrients can maximize microalgal growth, lipid accumulation, and overall biomass productivity (Zeng et al., 2011). Here are some strategies for optimizing carbon and nutrient uptake in microalgae for biofuel production:

- *Carbon Dioxide (CO<sub>2</sub>) Enhancement*: Increasing the availability of CO<sub>2</sub> can significantly improve microalgal growth and lipid production. This can be achieved through various methods such as the CO<sub>2</sub> enrichment method in which microalgal cultivation takes place in closed systems with controlled CO<sub>2</sub> concentrations which results in the enhancement of carbon assimilation (Barati et al., 2021). Techniques like flue gas utilization, carbon capture from industrial sources, or CO<sub>2</sub> supplementation from bicarbonate can provide additional CO<sub>2</sub> for microalgal cultivation. Another method is Microalgal Strain Selection in which microalgal strains are



identified and selected with high affinity for CO<sub>2</sub> uptake which contributes to improved carbon utilization efficiency.

- *Nutrient Optimization*: Microalgae require essential nutrients, including nitrogen (N), phosphorus (P), and micronutrients, for their growth and lipid accumulation. Optimizing nutrient availability can be achieved through various methods such as Nutrient Supplementation in which appropriate amounts of nutrients are provided to match the growth requirements of microalgae by Optimizing the N:P ratio in the growth medium that helpsto strike a balance between growth and lipid accumulation. Genetic engineering techniques can be also employed to enhance the nutrient uptake and utilization efficiency of microalgae (Zeng et al., 2011). Modifying genes involved in nutrient transporters or regulatory pathways can potentially enhance nutrient uptake and assimilation, thereby improving biomass productivity and biofuel production.

#### ***4.1.2 Enhancing lipid storage through genetic modification of Microalgae***

Enhancing lipid storage in microalgae can also be achieved by genetic engineering. Triacylglycerols, one type of lipid, are useful for making biofuels as well as for a number of industrial and dietary uses. The following techniques can be applied to improve lipid storage in microalgae via genetic engineering:

##### **4.1.2.1 Manipulating lipid synthesis pathways**

Overexpressing key enzymes involved in lipid synthesis pathways in microalgae can be a promising strategy for enhancing biofuel production. By increasing the expression levels of these enzymes, the production of fatty acids and triglycerides, which are the main constituents of lipid-based biofuels, can be significantly improved (Ran et al., 2019). Here are some examples of key enzymes that can be overexpressed in microalgae for biofuel production:

- *Acetyl-CoA Carboxylase (ACC)*: An essential precursor in the production of fatty acids, malonyl-CoA, is created when the enzyme ACC catalyzes the carboxylation of acetyl-CoA. Increased availability of malonyl-CoA can result in increased fatty acid synthesis in microalgae when ACC is overexpressed (Ran et al., 2019).

- *Fatty Acid Synthase (FAS)*: A group of enzymes known as FAS are in charge of creating long-chain fatty acids from acetyl- and malonyl-CoA. Increased lipid accumulation in microalgae is a result of the enzymatic machinery used in fatty acid production being enhanced by overexpression of FAS enzymes.
- *Diacylglycerol Acyltransferase (DGAT)*: DGAT is an enzyme that catalyzes the acylation of diacylglycerol (DAG) with fatty acids, which is the last step in the creation of triacylglycerol (TAG). The production of TAG can be increased by overexpressing DGAT, which also increases the overall lipid content of microalgae.
- *Phospholipid: Diacylglycerol Acyltransferase (PDAT)* is an additional enzyme necessary for the production of TAG. It aids in the buildup of TAG by catalyzing the transfer of fatty acids from phospholipids to DAG. In microalgae, overexpression of PDAT can increase lipid output and TAG synthesis.
- *Lysophosphatidic Acid Acyltransferase (LPAT)*: A key step in the manufacture of TAGs, phosphatidic acid (PA), is produced when lysophosphatidic acid (LPA) is acylated by the enzyme LPAT. The availability of PA can be increased by overexpressing LPAT, which encourages microalgae to produce TAG.
- *Oleosin*: Oleosins are tiny structural proteins that help to stabilize lipid droplets in microalgae cells. Oleosin overexpression can promote the creation of lipid droplets, shield lipids from oxidation, and boost lipid stability, all of which lead to greater lipid accumulation in microalgae.

#### 4.1.2.2 Downregulation of Competing Pathways

For microalgae to produce more lipids for biofuel, it is effective to downregulate competing pathways in lipid synthesis pathways. More resources can be directed toward lipid production by decreasing the activity or expression of processes that compete for carbon precursors or use metabolic intermediates (Khoo et al., 2023). Here are some examples of competing pathways that can be downregulated:

- *Starch Synthesis*: Particularly when there is an excess of photosynthetic carbon fixation, fat synthesis and starch biosynthesis compete for carbon precursors. ADP-glucose pyrophosphorylase (AGPase) and starch synthase are two genes that can be downregulated to limit the production of starch and divert carbon toward the synthesis of lipids (Ran et al., 2019).

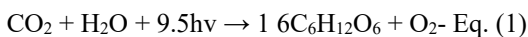
- *Protein Synthesis*: Amino acids derived from carbon and nitrogen sources are needed for protein synthesis but could also be used for lipid synthesis. Protein synthesis genes such as ribosomal proteins or amino acid biosynthesis enzymes can be downregulated to reduce protein synthesis and increase fat storage.
- *Respiratory Pathway*: For acetyl-CoA, a crucial precursor for the production of fatty acids, the respiratory route competes with lipid synthesis. It is possible to reduce the flux of carbon towards respiration and increase the amount of carbon available for lipid biosynthesis by down regulating genes implicated in the respiratory pathway, such as those encoding for enzymes in the electron transport chain or the tricarboxylic acid (TCA) cycle.
- *Triacylglycerol Degradation*: Triacylglycerols (TAGs), which are a type of lipid, are degraded into fatty acids by lipases, which are found in microalgae. The accumulation of stored lipids within the microalgae cells can be accelerated by downregulating genes linked to TAG degradation processes.

### 4.1.3 Enhancement of photosynthetic efficiency of microalgae

Microalgae can have their photosynthetic efficiency increased through genetic engineering, which will increase their capacity to transform light energy into chemical energy. Researchers can maximize the effectiveness of the photosynthetic process and boost biomass output by specifically targeting genes involved in photosynthesis. Here are some methods for genetically engineering microalgae to increase photosynthetic efficiency:

#### 4.1.3.1 Enhancing light capture and Utilization

By upregulating the expression of light-harvesting proteins such as phycobiliproteins or chlorophyll-binding proteins, microalgae can be genetically modified to absorb light more effectively. This enables them to capture a wider spectrum of light and use it for photosynthesis more effectively.



Eq. (1) states that as photon utilization in microalgae rises, light conversion efficiency rises and CO<sub>2</sub> fixation capacity rises as well. Numerous initiatives have been launched to increase photosynthetic efficiency and lessen photoinhibition's negative impact on microalgal

development. The majority of these aims to be shrink the size of the chlorophyll antenna (Zeng et al., 2011).

#### 4.1.3.2 Modifying photosynthetic pigments

Genetic engineering can change the type and quantity of photosynthetic pigments in microalgae. Enhancing the generation of biofuel by altering the photosynthetic pigments in microalgae can be a useful strategy. Chlorophyll and other photosynthetic pigments, such as carotenoids, are essential for harnessing light energy for photosynthesis. One method to improve light absorption and energy transfer effectiveness is to change the composition and amount of chlorophyll. For instance, optimizing light absorption at particular wavelengths can be achieved by increasing the ratio of chlorophyll a to chlorophyll b. In addition, increasing the efficiency of electron transfer in chlorophyll molecules can improve photosynthetic productivity as a whole. Carotenoids have various functions in photosynthesis, including light harvesting, photoprotection, and enhancing the efficiency of energy transfer. Modifying carotenoid composition and concentration can optimize light absorption and protect against photodamage. For example, enhancing the synthesis of carotenoids with high energy transfer efficiency, such as astaxanthin, can improve overall photosynthetic performance (Khosravitar, 2019).

#### 4.1.3.3 Optimizing carbon fixation

Genetic engineering can be utilized to improve the catalytic activity of important carbon fixation enzymes like ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), which is depicted in Figure 4. Speeding up carbon dioxide fixation and reducing the incidence of ineffective processes like photorespiration, can ultimately lead to an increase in photosynthetic efficiency.

#### 4.1.3.4 Enhancing electron transport and ATP Synthesis

The electron transport chain and ATP synthesis in microalgae can both be improved genetically. The total efficiency of energy conversion can be improved by altering the genes involved in these processes, such as those that encode electron transport proteins or ATP synthase subunits (Zeng et al., 2011).

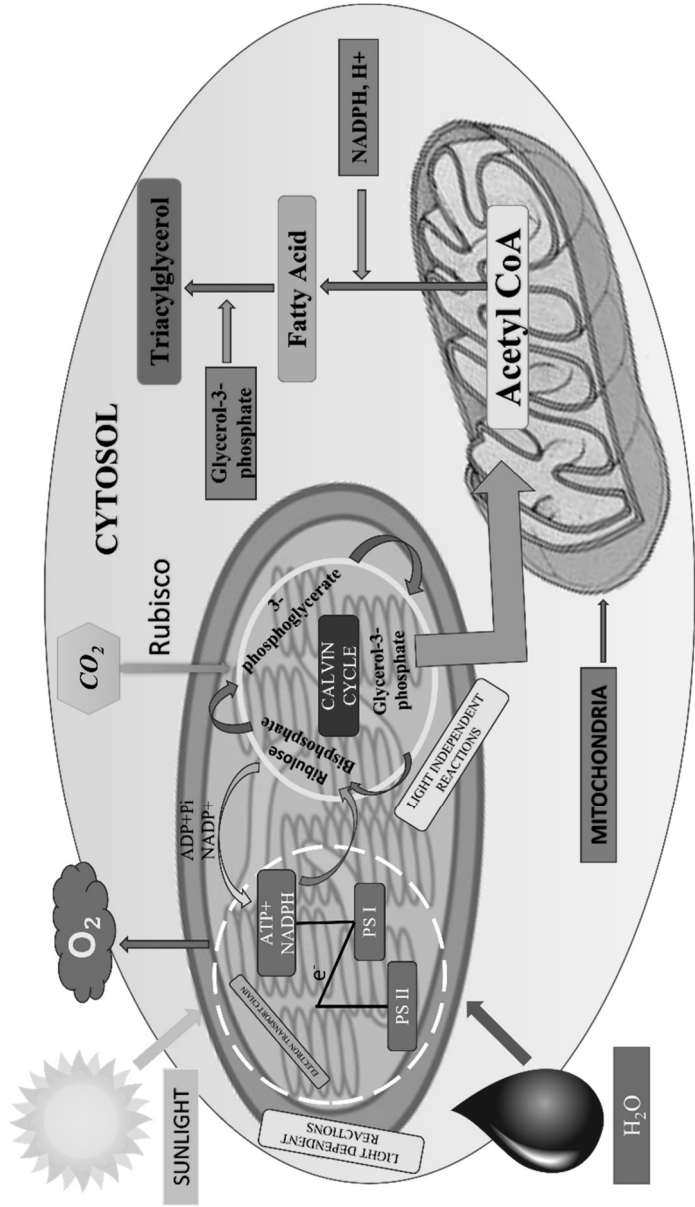


Figure 4: Schematic representation of photosynthesis, carbon dioxide fixation and lipid production for biofuel production.

#### 4.1.3.5 Improving CO<sub>2</sub> concentration mechanisms

Some microalgae have created efficient systems to concentrate and utilize carbon dioxide, such as the carbon concentrating mechanism (CCM). Genetic engineering can be used to enhance or add CCM components to microalgae that are deficient inefficient carbon concentration mechanisms. As a result, carbon dioxide can be efficiently absorbed and used by microalgae for photosynthesis (Khosravitar, 2019).

#### 4.1.4 Growing microalgae to produce biomass and trap carbon dioxide

It is a potential strategy that makes use of microalgae's photosynthetic capacity to generate biomass while reducing carbon dioxide emissions (Zeng et al., 2011). The following are some essential elements of microalgae cultivation for carbon dioxide capture and biomass production:

##### 4.1.4.1 Selection of suitable microalgae strains

Growth rates, biomass production, and CO<sub>2</sub> absorption abilities differ between microalgae strains. It is crucial to choose strains that are compatible with the particular growing circumstances and have traits that are advantageous for biomass production and CO<sub>2</sub> capture. The strains *Chlorella*, *Spirulina*, and *Nannochloropsis* are a few that are frequently used.

##### 4.1.4.2 Cultivation systems

Microalgae can be cultivated in various systems, including open ponds, closed photobioreactors, and raceway ponds. The choice of cultivation system depends on factors such as available land, climate, required scale, and control over environmental parameters. Each system has its advantages and challenges in terms of biomass productivity, CO<sub>2</sub> utilization efficiency, and operational costs. Microalgal cultivation systems may be carefully managed and controlled, which is significant. For optimum biomass and oil yields, temperature, pH, nutrient, and carbon dioxide levels can be monitored and optimized (Singh et al., 2019). Sunlight energy is converted into chemical energy by microalgae. CO<sub>2</sub> is simultaneously transported to carbon-containing substances like proteins, lipids, and carbohydrates and fixed. As a result, the biomass of microalgae reflects the ability to fix CO<sub>2</sub> (Zeng et al., 2011).