

Utilizing Bioreactors for Enhancing Biofuel and Biochemical Production from Microorganisms

¹Mustafa R. AL-Shaheen , ²Ghassan.F. Al.Samarrie

Department of Biotechnology, College of Science, University of Anbar, Anbar, Iraq

Department of Biology, College of Education, University of Samarra, Samarra, Iraq

Abstract

Bioreactors present an innovative approach for improving biofuel and biochemical production from microorganisms. This technology relies on the use of living microorganisms, such as bacteria and fungi, to convert organic materials into energy and valuable chemical products. Bioreactors offer several advantages. They operate with high efficiency and require smaller spatial footprints compared to traditional methods of biological production. Additionally, they reduce harmful emissions and serve as a sustainable alternative to fossil fuel-dependent processes. Bioreactors are employed in the production of biofuels, such as animal-based and plant-based biofuels, which can be utilized as alternative energy sources. They are also utilized for the production of bio-based chemicals, such as organic materials.

Keywords: Bioreactors, Biofuel production, Biochemical production, Microorganisms, Organic materials

1. INTRODUCTION

1.1. Definition of bioreactors

Bioreactors are crucial in various industries, including pharmaceuticals, food and beverage, agriculture, waste management, and biofuel production. They create optimal conditions for the growth and maintenance of living cells or organisms. In the context of biofuel and biochemical production, bioreactors are essential for harnessing the power of living systems and meeting the increasing demand for renewable energy sources while reducing greenhouse gas emissions. There are two types of bioreactors used in biofuel production: open and closed systems. Open systems like raceway ponds are cost-effective for large-scale microbial biomass production, while closed bioreactors are necessary for providing inoculum cultures. However, the cost of manufacturing and operating closed bioreactors can be restrictive, so ongoing research focuses on optimizing the cost-benefit ratio. Research is also being conducted on converting carbon dioxide (CO₂) in closed bioreactors to utilize it as a resource for biofuel production and contribute to carbon mitigation efforts. Additionally, advancements have been made in improving energy conversion efficiency within bioreactor systems through artificial leaves and novel designs [1],[2].

Microalgae-based biorefinery concepts show promise in biofuel and biochemical production. Microalgae offer advantages such as high growth rates, efficient carbon utilization, elimination of food industry competition, and cultivation on degraded land. Genetic engineering techniques enable the

production of valuable compounds using microalgae. To achieve high yields of biofuels and other valuable products, culture systems must be optimized for high lipid content, primary productivity, and biomass growth rates. The design and size of bioreactors can vary depending on specific requirements, ranging from laboratory flasks to large-scale raceway ponds. Closed bioreactors have shown to be more productive than open systems. Different industries require specialized bioreactors tailored to their needs. For example, bioreactors used for producing toxic products differ from those used for biofuel production. Overall, bioreactors are essential tools in biofuel and biochemical production, providing a controlled environment for optimizing production and contributing to a carbon-neutral economy while reducing greenhouse gas emissions. See references [3], [4].

1.2. Importance of enhancing biofuel and biochemical production from microorganisms

The importance of enhancing biofuel and biochemical production from microorganisms cannot be overstated in the pursuit of a sustainable bioeconomy. Biofuels, derived from biological material, offer a renewable alternative to fossil fuels and play a crucial role in reducing greenhouse gas emissions and mitigating climate change. While there are different generations of biofuels, with the fourth generation just emerging, utilizing bioreactors for biofuel production is an essential strategy. Bioreactors are specialized vessels that provide a controlled environment for the growth and multiplication of microorganisms, plant cells, or animal cells [5]. They have revolutionized the production of bioproducts such as enzymes, antibiotics, and biofuels on an industrial scale. In the context of biofuel production, bioreactors offer numerous advantages. They enable efficient cultivation of microorganisms for optimal biomass growth and metabolism. With their precise operational control for multiphase flow and heat and mass transfer, bioreactors can improve energy conversion efficiency. Additionally, bioreactors have proven to be crucial in microbial biofuel conversion processes. They are widely used in various processes such as anaerobic digestion for biogas production, photo-fermentation or dark-fermentation for hydrogen production, fermentation for alcohol production, and microalgae cultivation for fatty acid production. By providing an ideal milieu for microbial growth and metabolism, bioreactors ensure stable conditions necessary for efficient biofuel synthesis [6].

In order to maximize biofuel production, it is essential to find suitable microorganisms that can completely utilize sugars derived from lignocellulosic biomass feedstock while tolerating inhibitory compounds generated during pretreatment. The ideal strain should exhibit high metabolic fluxes and biosynthesize single fermentation products through fast pathways. Metabolic engineering plays a crucial role in developing such strains by conferring them with the necessary genes for high cell mass growth and enhanced tolerance to inhibitors. Furthermore, pond-grown microalgae offer significant promise as feedstocks for biofuel production. Compared to conventional terrestrial biofuel crop production, pond-based algal biofuel production requires significantly less land area and offers additional ecological benefits [7]. It reduces anthropogenic pollutant releases and requires lower water subsidies, making it a more sustainable option. To enhance biofuel and biochemical production from microorganisms, it is necessary to understand the metabolism of photosynthetic organisms such as cyanobacteria and algae. Genetic engineering and efficient cultivation techniques, along with increased CO₂ assimilation, can enable the production of high-energy chemical products from renewable resources. In conclusion, enhancing biofuel and biochemical production from microorganisms is of paramount importance in our transition towards a sustainable bioeconomy.

Bioreactors play a crucial role in providing an optimal environment for microbial growth and metabolism. By utilizing bioreactors and focusing on metabolic engineering, we can unlock the full potential of microorganisms for efficient and sustainable biofuel production. This will contribute to reducing greenhouse gas emissions, mitigating climate change, and ensuring a secure energy future. See references [8], [9].

2. OVERVIEW OF BIOREACTORS

2.1. *Description of the technology*

Bioreactors are versatile vessels that play a crucial role in various industries. They provide an ideal environment for the growth and maintenance of living cells or organisms, allowing for efficient production processes. In the field of industrial biotechnology, bioreactors have revolutionized the production of bioproducts like enzymes, antibiotics, and biofuels. They enable the large-scale growth of microorganisms, plant cells, or animal cells to obtain products that were previously difficult to produce. In biofuel production, bioreactors are key technologies. They offer a controlled environment for the growth of microorganisms that convert renewable resources into biofuels. This sustainable approach addresses the need for alternative energy sources and reduces greenhouse gas emissions. However, improving the efficiency of photon-to-fuel conversion is a challenge. Ongoing research aims to develop higher conversion efficiencies through advancements in biomass processing and better feedstocks[10]. Bioreactors also play a crucial role in microbial biofuel conversion processes. They provide an optimal environment for microbial growth during fermentation processes that generate biofuels from various substrates. The specifications of bioreactors need to integrate structural configuration with operational control to optimize flow and transfer within the reaction solution. Biorefineries are emerging as integrated systems that utilize biomass for sustainable production. Bioreactors are vital in these facilities, providing an ideal environment for microbial growth and product synthesis. However, challenges remain, such as the separation of different fractions in microalgae-based biorefineries. Research aims to develop cost-effective processes that utilize microalgae to produce high-value compounds alongside biofuels. The use of membrane bioreactors is being explored to improve biofuel production efficiency. In conclusion, bioreactors are essential tools in biotechnology and have revolutionized industries. In biofuel production, they enable efficient and sustainable processes. Ongoing research focuses on improving conversion efficiency and developing higher-value compounds. Bioreactors integrated into biorefinery concepts enhance their potential. Despite challenges, membrane bioreactors show promise for advancing biofuel production technologies in the future. See references: [11], [12].

2.2. *Role of living microorganisms in the process*

The role of living microorganisms in bioreactors is essential for efficient biofuel and biochemical production. Microbial biofuels, derived from prevalent raw materials and mild operation conditions, offer promising alternatives to conventional fuels. Bioreactors create an optimal environment for microbial growth and metabolism, allowing for maximum biofuel production. These reactors, especially closed systems, provide the ideal milieu for microbial cells to thrive. Bioreactors play a crucial role in both the upstream and downstream processes of microbial biofuel conversion. In the upstream process, fermentation is used to promote microbial growth and product generation. The

bioreactor ensures that the correct structural configuration and precise operational control are maintained to optimize multiphase flow, as well as heat and mass transfer within the reaction solution. Different types of bioreactors are widely used in various microbial biofuel conversion processes. Anaerobic digestion bioreactors produce biogas, while photo-fermentation or dark-fermentation bioreactors generate hydrogen. Fermentation bioreactors are used for alcohol production, and microalgae-based bioreactors produce fatty acids. Microbes utilize a variety of substrates such as cellulose, hemicellulose, starch, glucose, and xylose to produce biofuels [13], [14].

However, maintaining stability in microbial biofuel conversion processes is crucial since microbial cells are sensitive to variations in their surroundings. Any instability can be detrimental to their growth and product synthesis. Therefore, it is necessary to integrate precise operational control within the bioreactor specifications to ensure optimal energy conversion efficiency. While there are several advantages to utilizing bioreactors for biofuel production, one significant limitation is its implication on the energy-water-food nexus. The increasing demand for biofuels has created direct competition with global food resources and land availability. Furthermore, large amounts of water and energy are required for crop harvesting to generate feedstocks for first-generation biofuels. To address these limitations, there is ongoing research in the development of third and fourth-generation biofuels. These biofuels offer environmentally friendly and sustainable alternatives, but the research process is time-consuming and requires expert handling. In summary, bioreactors play a vital role in maximizing biofuel production by creating an optimal environment for microbial growth and metabolism. They are widely used in various microbial biofuel conversion processes. While there are challenges related to the energy-water-food nexus, careful management can ensure that biofuel production remains environmentally advantageous and reliable for sustainable development. With further scientific breakthroughs and advancements in metabolic engineering, the feasibility and efficiency of biofuel production can be enhanced, making it a promising solution for a carbon-neutral bioeconomy. See references [15], [16], [17].

3. ADVANTAGES OF BIOREACTORS

3.1. *High efficiency*

Bioreactors offer numerous advantages that contribute to their high efficiency in various applications. These versatile vessels provide the ideal conditions for the growth and maintenance of living cells or organisms, allowing for efficient and reliable production. In the context of bioreactor applications, they are used in industries such as pharmaceuticals, food and beverage, agriculture, and waste management. Bioreactors play a crucial role in the field of industrial biotechnology, enabling the production of bioproducts like enzymes, antibiotics, and biofuels. They create a controlled environment that facilitates the growth and multiplication of microorganisms, plant cells, or animal cells on a large scale. This revolutionizes the production of products that were previously challenging to obtain industrially. For example, bioreactors have greatly improved biofuel production by enabling high yields, volumetric productivities, and titers [18], [19]. They have also facilitated the extraction of lipids from algae with increased efficiency by eliminating energy-intensive drying processes. Additionally, bioreactors can be utilized for hydrogen production from microalgae through biological photolysis using sunlight and water. Despite challenges such as oxygen generation during biological hydrogen production or lignocellulose degradation in biofuel synthesis, future trends in microbial

biofuel production hold promise through genetic engineering and metabolic engineering techniques. Overall, bioreactors offer immense potential for high-efficiency biofuel and biochemical production while minimizing waste accumulation and mitigating greenhouse gas emissions. See references [20], [21].

3.2. Small spatial footprints

Bioreactors are advantageous for biofuel and biochemical production due to their small spatial footprints, making them efficient in terms of space utilization. Microalgae, commonly used in bioreactors, have high cell growth rates and can convert solar energy into chemical energy efficiently. They require aquatic environments and can be cultivated using water and atmospheric carbon dioxide, reducing the need for arable land. Additionally, microalgae have the potential to be used for other valuable products such as food supplements, cosmetics, and pharmaceuticals. Advancements in biomass processing technologies and better feedstocks contribute to higher photon-to-fuel conversion efficiencies (PFCE) in bioreactors. Fourth generation biofuels aim to achieve even higher PFCEs by utilizing photosynthetic microorganisms as catalysts for fuel production. Optimizing the performance of bioreactors and achieving higher PFCEs require appropriate configuration. Research on "designer organisms" aims to develop microorganisms with 10% PFCE. Immobilization of cyanobacteria and algae may help achieve this goal. In conclusion, utilizing bioreactors offers the advantage of small spatial footprints and allows for efficient use of land resources while achieving higher PFCEs. Ongoing research and advancements in biomass processing technologies make bioreactors increasingly important in sustainable biofuel production. See references [22], [23].

3.3. Reduction of harmful emissions

Bioreactors offer advantages in biofuel and biochemical production, including reduced emissions. Biofuels, derived from biological sources, are renewable and help decrease greenhouse gas emissions. Developing efficient strategies for solar-based biofuel production is important for a sustainable bioeconomy, especially in regions with limited resources like the EU. Biofuels are crucial in the transportation sector and have become the largest renewable fuel globally. Improving photon-to-fuel conversion efficiency (PFCE) is a key aspect of biofuel production. Ongoing research aims at higher PFCEs through advancements in biomass processing and the use of better feedstocks. Fourth generation biofuels, utilizing synthetic biology, aim to produce high-quality fuel using photosynthetic microorganisms. New eco-friendly trends in biofuel production involve using microorganisms to convert diverse crude materials into renewable and environmentally friendly biofuels. Overall, utilizing bioreactors contributes to reducing emissions and addresses environmental concerns while promoting energy security and economic viability in the face of limited oil reserves. See references: [24], [25].

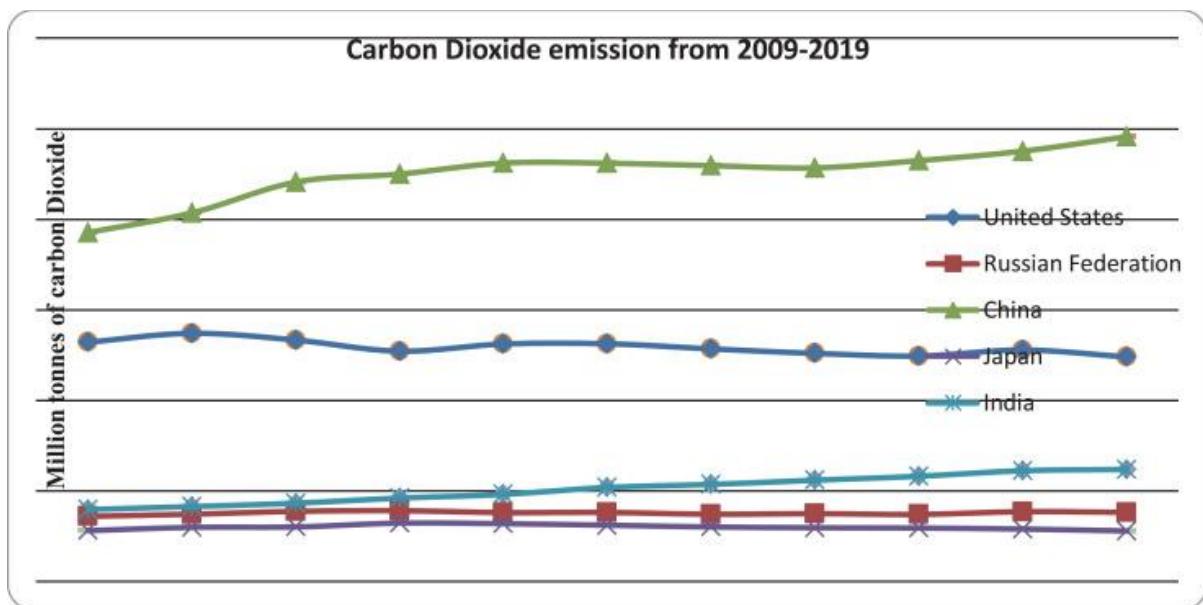


Figure 1: Top countries with higher rate of carbon dioxide emission. Source: [26].

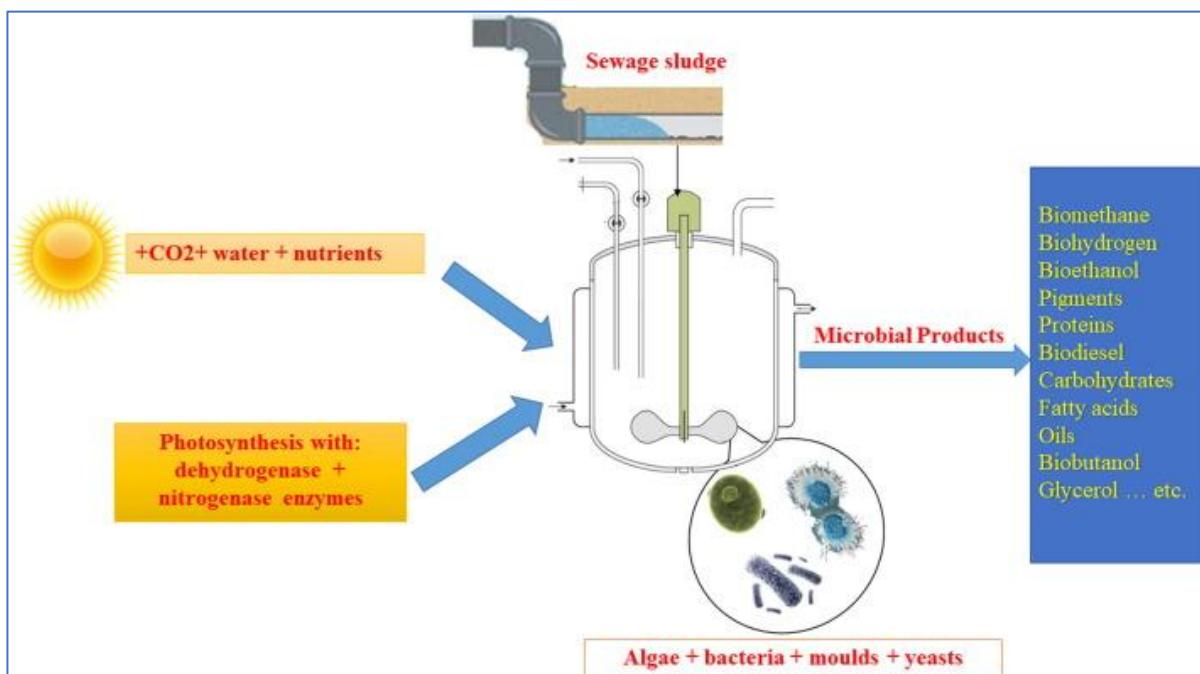


Figure 2: Substrate and sources for biofuel production. Sources [27].

3.4. SUSTAINABILITY COMPARED TO FOSSIL FUEL-DEPENDENT PROCESSES

Utilizing bioreactors for biofuel and biochemical production has several advantages over fossil fuel-dependent processes. Biofuels are derived from biological material and have a renewable origin through photosynthetic conversion of solar energy. Unlike fossil fuels, which rely on ancient photosynthesis, biofuels are based on present-day photosynthesis, making them more sustainable. Biofuels can be classified into different generations based on their origin and production technology. Second-generation biofuels maximize the use of plant-based resources, while third-generation biofuels focus on algal biomass production, requiring less land area. Fourth-generation biofuels, known as

photobiological solar fuels and electrofuels, directly convert solar energy into fuel using widely available raw materials. Synthetic biology plays a crucial role in this development by enabling the discovery of new solutions and the construction of synthetic living factories for efficient energy conversion. Bioprospecting microbial strains has proven effective in enhancing biofuel production efficiency. Microbes naturally produce bioactive compounds that can be used as fuels through metabolic engineering. Ongoing studies aim to increase biofuel production while reducing costs for sustainable industries. Biofuels offer significant environmental and energy security advantages over fossil fuels. They reduce greenhouse gas emissions, alleviate climate change, and provide economic improvements. Lignocellulosic biomass is a reliable feedstock for renewable energy production without competing with food sources. The utilization of plant-based resources and microbial bioprocessing techniques further enhance the sustainability and potential of biofuel production. In conclusion, bioreactors offer numerous advantages for biofuel and biochemical production compared to fossil fuel-dependent processes. See references: [28],[29].

4. APPLICATIONS OF BIOREACTORS IN BIOFUEL PRODUCTION

4.1. *Animal-based biofuels*

Animal-based biofuels play a significant role in the application of bioreactors in biofuel production. With the global rise in energy consumption and the increasing concerns about sustainability and environmental impact, there is a renewed interest in biofuel sectors as an alternative to crude oil. The combustion of crude oil leads to harmful greenhouse gas emissions, contributing to air pollution and climate change. Bioenergy technologies, including the use of animal-based biomass, offer a solution to these issues by utilizing microorganisms to manufacture various types of biofuels. Biofuels are renewable fuels that acquire their energy from biological carbon dioxide fixation. Unlike fossil fuels, biofuels are biodegradable and environmentally friendly. They can be derived from live animals or by-products that are less than 20 years old, making them a sustainable energy source. Animal leftovers, agricultural waste, and oil crops are among the diverse crude materials being explored for biofuel production. One promising approach in biofuel production is through the utilization of bioreactors. Bioreactors provide controlled environments for microorganisms to produce biofuels efficiently. These reactors can be designed to accommodate different types of microorganisms and optimize their growth conditions. Additionally, bioreactors allow for continuous production and scaling up of biofuel production [30],[31].

The future trends in microbial biofuel production include genetic engineering of microorganisms for increased product specificity and higher-level biofuels. The focus will also be on lignocellulose degradation, which is crucial for efficient conversion of biomass into biofuels. Metabolic engineering plays a vital role in enhancing microbial biofuel production by manipulating metabolic pathways and catalytic enzymes. It involves introducing imperative genes or enzymes extracted from efficient biofuel-producing organisms into non-biofuel producing microorganisms. Microalgae are another promising source for biodiesel production using animal-based biomass. These photosynthetic organisms can be cultivated using water and atmospheric carbon dioxide, reducing production costs compared to other organisms. Furthermore, microalgae can be genetically modified to produce various compounds, including non-native products. They have high photosynthetic efficiencies and can be grown in degraded land, eliminating the controversies of food vs. Fuels [32].

In conclusion, animal-based biofuels and the utilization of bioreactors are integral to the production of biofuels. They offer renewable and environmentally friendly alternatives to crude oil while addressing concerns about sustainability and economics. With advancements in genetic engineering and metabolic engineering, microbial biofuel production is expected to increase in productivity and affordability. Similarly, microalgae-based biodiesel production holds great potential for various applications beyond biofuels. These developments highlight the importance of utilizing animal-based biomass and bioreactors in achieving a bio-based economy and transitioning towards cleaner and sustainable energy sources. See references: [33].

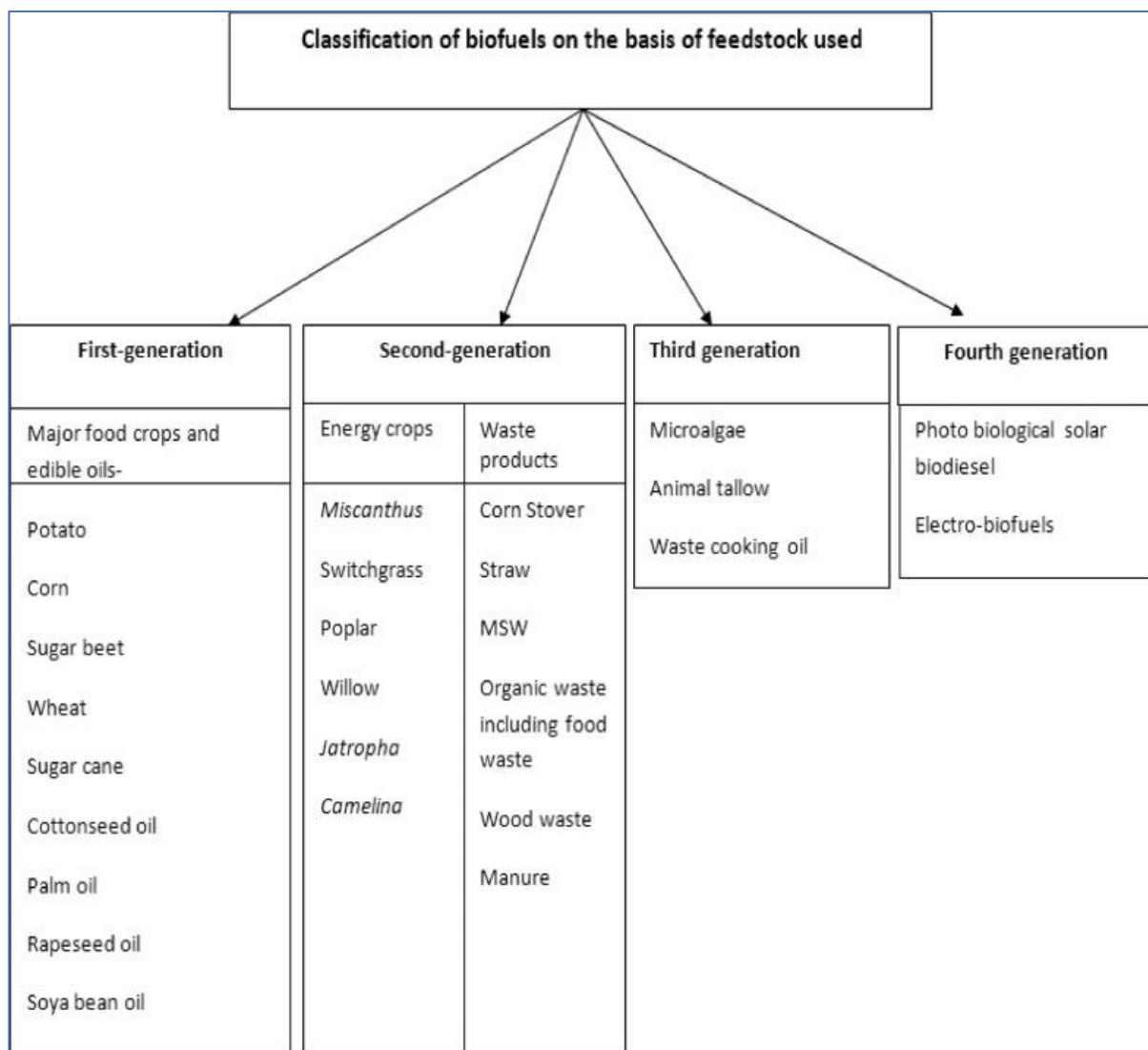


Figure3: Classification on the biofuels on the basis of feedstock used for production. Source [34].

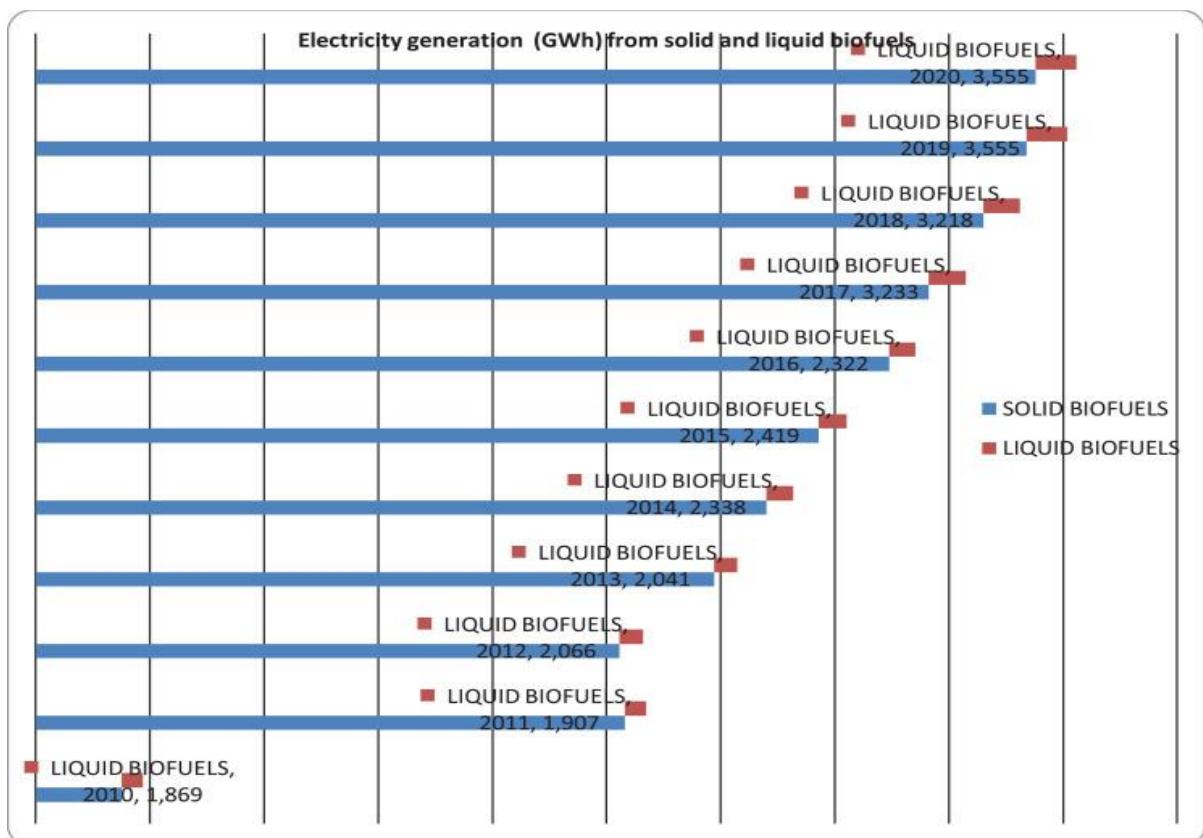


Figure 4: Utilization of solid and liquid biogas in the past years worldwide for electricity generation. Source:[35].

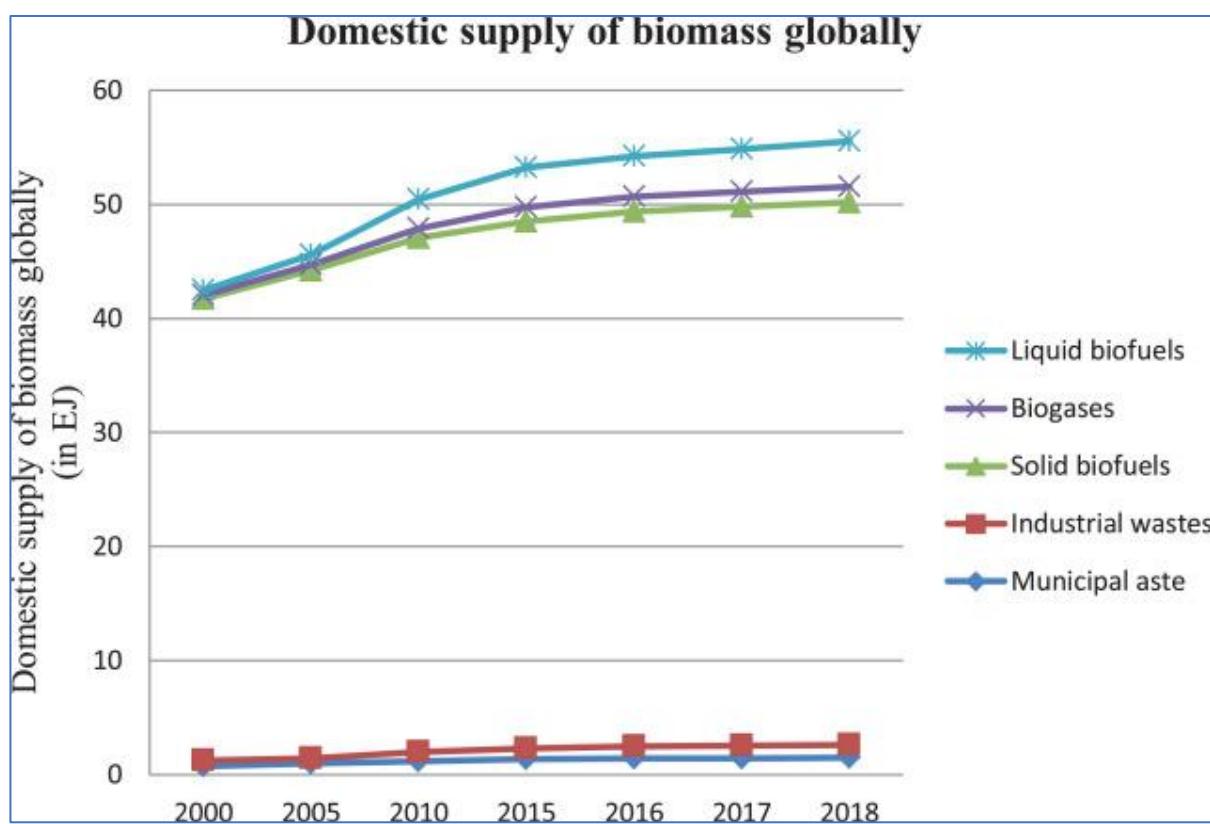


Figure 5: Domestic supply of biomass globally. Source: [36].

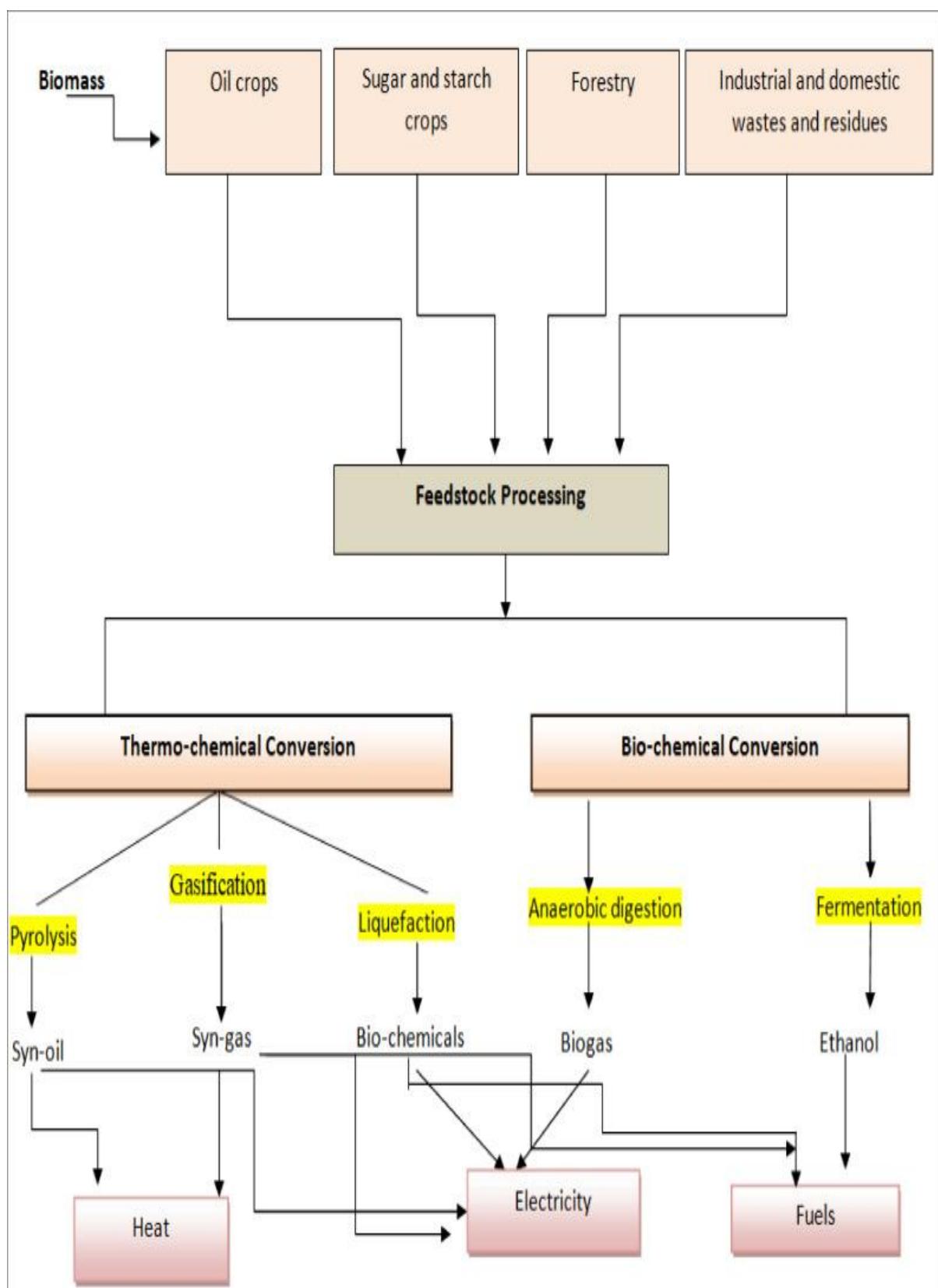


Figure 6: Overview of major biomass conversion. Source: [37]

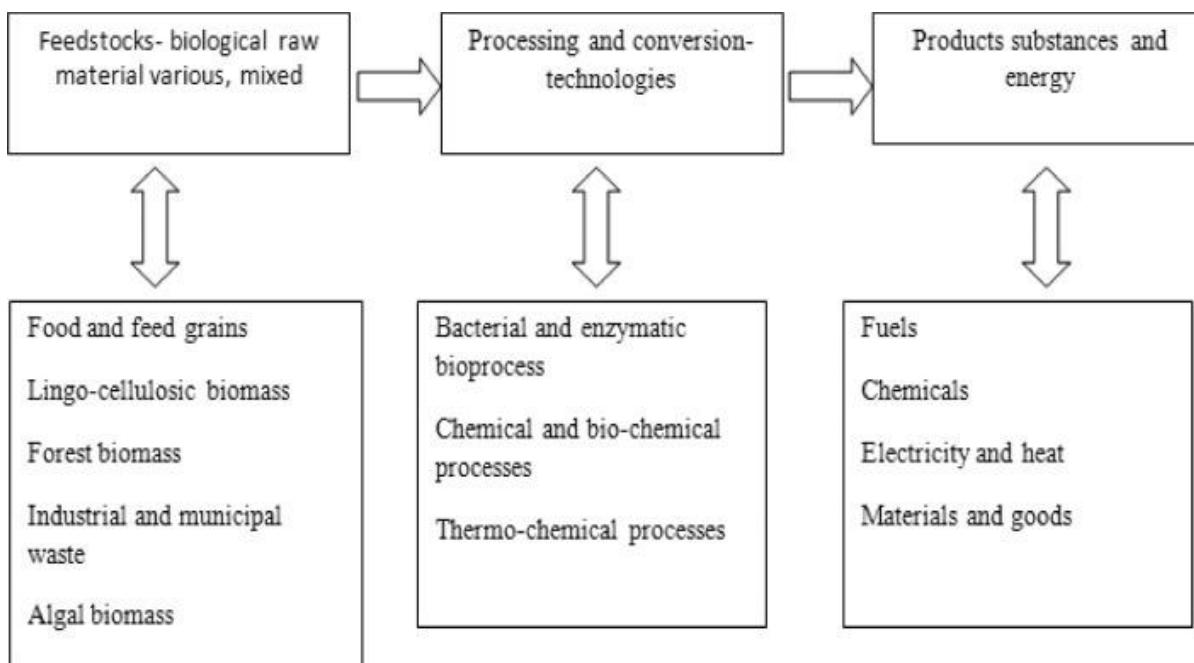


Figure 7: Overview of basic principles of biorefinery. Source: [38]

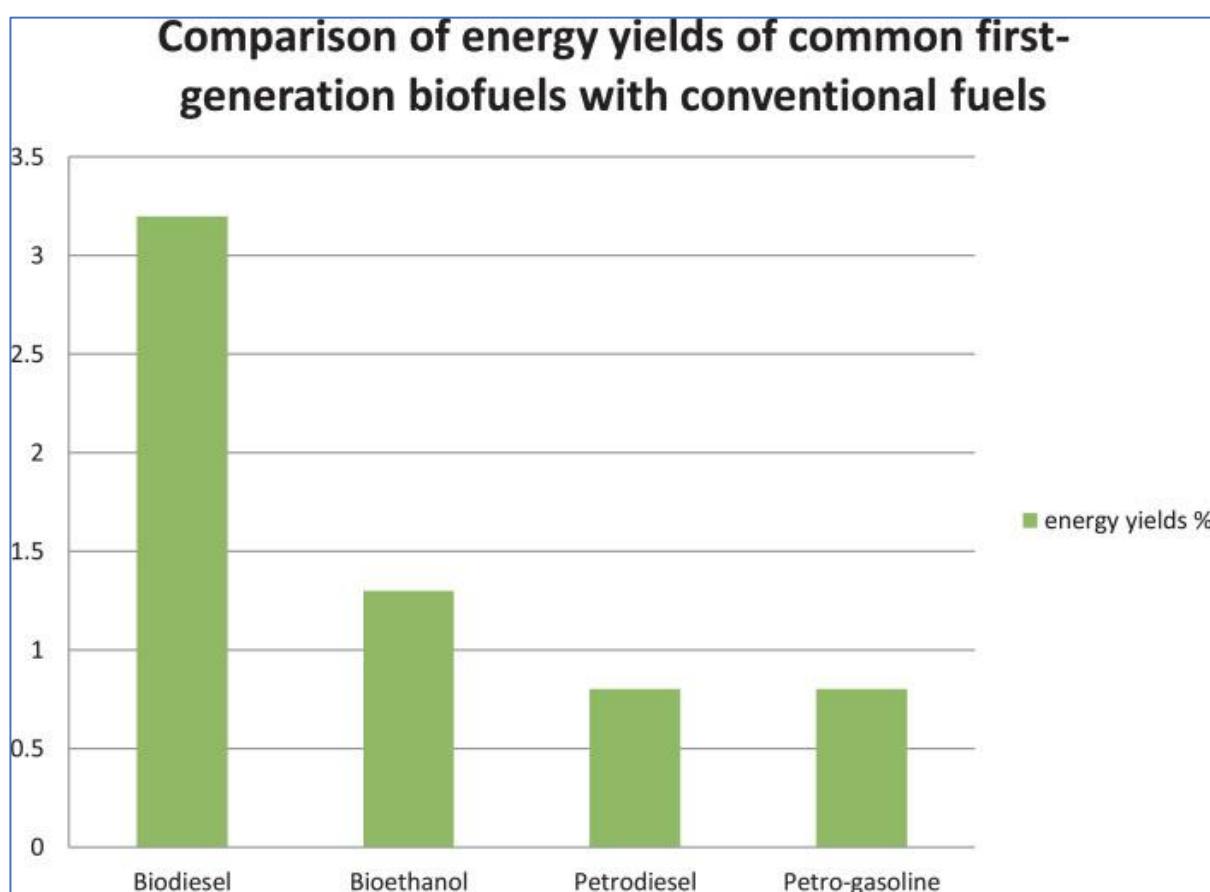


Figure 8: The comparison between biodiesel/bioethanol with petrodiesel/petro-gasoline on the basis of their overall energy yields. Source: [39]

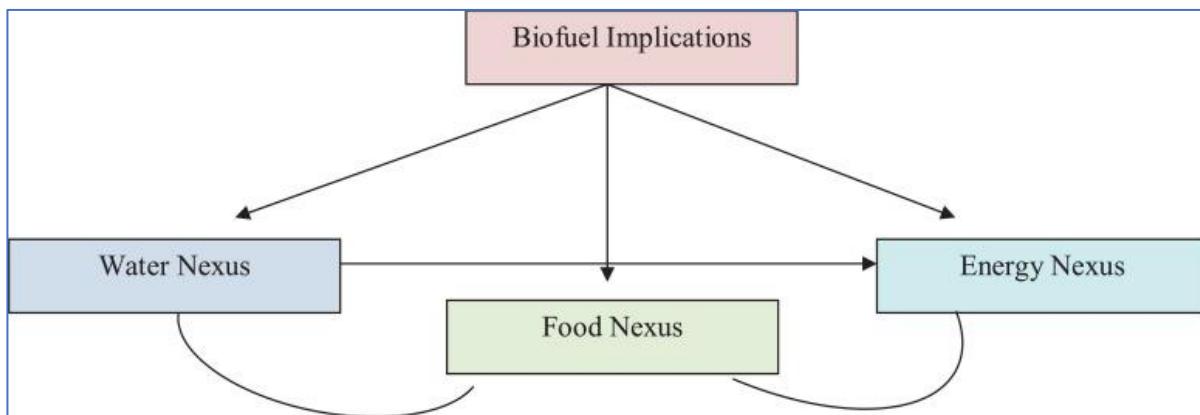


Figure 9: Biofuels implications and its interdependency on food, water and energy nexus.
Source:[40].

4.2. Plant-based biofuels

Utilizing bioreactors for biofuel production is a promising solution to meet the global energy demand while reducing environmental impact. Plant-based biofuels derived from lignocellulosic biomass offer a reliable feedstock without competing with food sources. Unlike first-generation biomass, these plant sources do not compromise food availability. Producing biofuels from lignocellulosic feedstock reduces dependence on fossil fuels and provides economic benefits to rural communities. Efficient conversion of biomass into biofuels requires suitable microorganisms that can effectively utilize sugars produced from the feedstock. These organisms should also tolerate inhibitory compounds generated during the production process. Efforts are being made to develop natural cellulolytic microorganisms or engineer industrial strains with the necessary genes for biofuel production. Metabolic engineering techniques can optimize these strains for large-scale production [41].

Bioreactors play a crucial role in converting biomass into biofuels by providing favorable conditions for microorganism growth and metabolic activity. Operating at high temperatures improves reaction rates and reduces contamination risk. Controlling temperature and pH levels minimizes interference from other microbes. In conclusion, utilizing bioreactors for biofuel production offers a promising solution to the increasing global energy demand while reducing environmental impact. Plant-based biofuels derived from lignocellulosic biomass have advantages over first-generation feedstocks. Finding suitable microorganisms and optimizing their genetic and metabolic characteristics are key challenges. Bioreactors provide controlled environments for efficient biofuel production. Advancements in metabolic and process engineering are helping increase biofuel production while reducing costs [42].

5. APPLICATIONS OF BIOREACTORS IN BIOCHEMICAL PRODUCTION

5.1. Production of organic chemicals

The production of organic chemicals for applications in bioreactors for biochemical production is an important area of research and development. Biofuel and biochemical production by photosynthetic microorganisms, such as cyanobacteria and algae, have gained attention due to their potential to improve energy security and reduce CO₂ emissions. These photosynthetic microorganisms offer the

advantage of utilizing renewable and potentially carbon-neutral resources, making them attractive for biofuel production [43]. One of the key challenges in this field is the need for significant innovation to make biofuel production by photosynthetic microorganisms feasible in practice. However, these fuels hold great promise due to their ability to grow on non-arable land and utilize saline and wastewater streams. In order to capture solar energy and convert atmospheric and waste CO₂ into high-energy chemical products, it is crucial to have a deep understanding of the metabolism of these organisms. Metabolic engineering plays a vital role in enhancing the feasibility of biofuel and biochemical production. By manipulating the metabolic pathways of photosynthetic organisms, researchers can optimize their ability to convert CO₂ into valuable chemical products. Additionally, efficient cultivation techniques must be developed to maximize biomass production and ensure consistent yields [44]. Downstream processing is another critical aspect that needs to be addressed in order to achieve commercial-scale production. Biofuel production processes often yield co-products such as animal feed, heat, electricity, and biochemicals. Allocating these co-products correctly is essential when assessing the environmental impacts of biofuel production. Various methods can be used for allocation, including system expansion or using allocation factors based on physical or economic relationships. Furthermore, lignocellulosic biomass serves as a reliable feedstock for renewable energy production. Unlike first-generation biomass feedstocks that compete with food sources, lignocellulosic feedstocks are abundant in nature and globally available. The production of biofuels from lignocellulosic feedstocks has been proven to be environmentally friendly and economically beneficial, especially for rural communities [45].

Microbes play a crucial role in biofuel production, as they can produce bioactive compounds and enzymes that act on diverse feedstocks. However, the high cost of enzymes remains a challenge in the development of an economically feasible lignocellulose-based biofuel industry. Nonetheless, ongoing research in genetic engineering and optimization of fermentation parameters is focused on increasing biofuel production while reducing production costs. In addition to biofuels, microalgae also have the potential to produce high-value compounds such as pigments, microelements, omega fatty acids, and antioxidants. However, downstream processing and purification of these compounds remain challenging. To achieve economic feasibility in commercial-scale production, proper biomass production conditions must be designed to maximize targeted compound yields, and high-energy-requiring processes should be eliminated. To optimize the biorefinery approach that integrates biofuel and other value-added products from microalgae, it is essential to reduce the number of steps involved in the production process. Light and temperature sensitivity of certain high-value products must also be addressed during separation processes. Moreover, research into the biology of cells and their metabolites should be expanded to explore more value-added products [46], [47].

Looking towards the future, microbial factories hold great potential for biofuel production. The selection of efficient microbes and substrates is crucial for synthesizing biofuels with positive net energy balance. Lignocellulosic-containing substrates are desirable alternatives due to their abundance and non-competition with food sources. However, some microorganisms still struggle with complete lignocellulose degradation. In order to maximize renewable carbon and hydrogen conversion from second-generation biomass, ongoing projects are focusing on improving metabolic fuel production and separation processes in bio-oil production. The emergence of fourth-generation biofuels based on photobiological solar fuels and electrofuels shows promise for fundamental breakthroughs in this field.

Synthetic biology is expected to play a crucial role in developing synthetic living factories and designer microorganisms for efficient conversion of solar energy into fuel[48].

In conclusion, utilizing bioreactors for biofuel and biochemical production holds significant potential for improving energy security and reducing CO₂ emissions. The metabolic engineering of photosynthetic microorganisms, efficient cultivation techniques, and downstream processing are key areas of focus for enhancing the feasibility of biofuel production. Additionally, the utilization of lignocellulosic feedstocks and the development of microbial factories offer exciting opportunities for sustainable fuel alternatives. Continued research and development in these areas will be essential for advancing the field of biofuel and biochemical production. See references: [49].

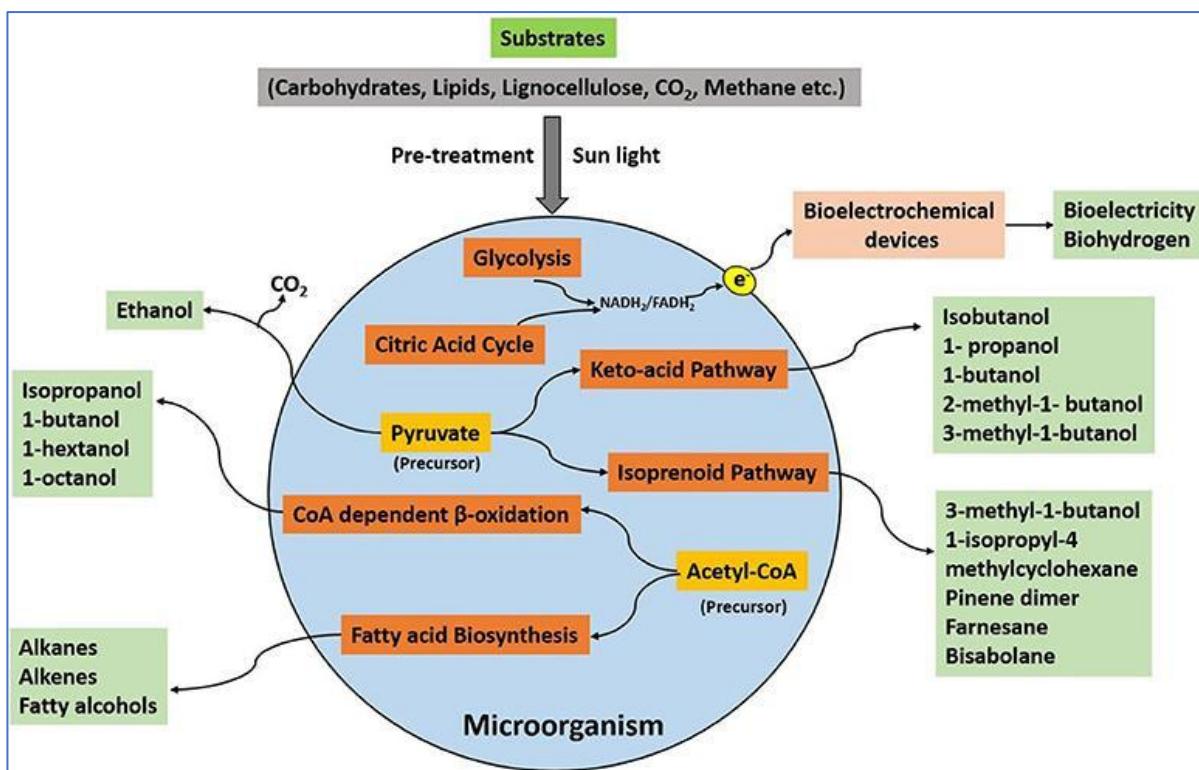


Figure 10 : An overview of microbial metabolic pathways for biofuel production. Source: [50]

6. CONCLUSION

In conclusion, the utilization of bioreactors for biofuel and biochemical production presents a promising and economically viable alternative to conventional fuel sources. The increasing production of biofuels from edible sources demonstrates their stability and potential in meeting energy demands. However, it is important to address the limitations associated with first-generation biofuels, such as their involvement in the food chain and competition with food production. Additionally, the high production cost and land-use efficiency issues need to be carefully managed. Second-generation biofuels, derived from non-edible lignocellulosic feedstocks, offer advantages such as not interfering with food production and higher production per unit land area. However, the more sophisticated instrumentation required for their production necessitates increased investment.

6. REFERENCES

1. Dao, X., and Wu, Y. (2021). Artificial leaf for light-driven CO₂ reduction: Basic concepts, advanced structures and selective solar-to-chemical products. *Journal of Hazardous Materials*, 410, 125-193. <https://doi.org/10.1016/j.jhazmat.2020.125193>.
2. Wang, L., Chen, H., and Zhang, Z. (2023). Ingenious artificial leaf based on covalent organic framework membranes for boosting CO₂ photoreduction. *Journal of the American Chemical Society*, 145(17), 9520-9529. <https://doi.org/10.1021/jacs.2c11146>.
3. Joronen, T., Nymalm, M., 6 Räisänen, M.-L. (2020). Solar-powered carbon fixation for food and feed production using microorganisms *A comparative techno-economic analysis*. *ACS Omega*, 5(30), 19234–19247. <https://doi.org/10.1021/acsomega.0c04926>.
4. Moreira, C., Cavaco-Paulo, A., and Serafim, L. S. (2022). Kinetic modeling of CO₂ biofixation by microalgae and optimization of carbon supply in various photobioreactor technologies. *ACS Sustainable Chemistry and Engineering*, 10(38), 12826-12842. <https://doi.org/10.1021/acssuschemeng.2c03927>.
5. Nocera, D. G. (2012). The artificial leaf. *Accounts of Chemical Research*, 45(5), 767–776. <https://doi.org/10.1021/ar2003013>.
6. Posten, C., and Walter, C. (2016). Lipid production with *Trichosporon oleaginosus* in a membrane bioreactor using microalgae hydrolysate. *Journal of Hazardous Materials*, 320, 279-290. <https://doi.org/10.1016/j.jhazmat.2016.279841>.
7. Lee, G. Y., Yoo, C., Jun, S. Y., Ahn, C. Y., and Oh, H. M. (2015). Lipid production of microalga *Chlorella sorokiniana* CY1 is improved by light source arrangement, bioreactor operation mode and deep-sea water supplements. *Biotechnology and Bioengineering*, 112(7), 1296–1305. <https://doi.org/10.1002/biot.201500288>.
8. El-Sheekh, M. M., and El-Zeiny, A. E. (2022). Maximizing nitrogen removal and lipid production by microalgae under mixotrophic growth using response surface methodology: *Towards enhanced biodiesel production*. *Fermentation*, 8(12), 682. <https://doi.org/10.3390/fermentation8120682>.
9. Rodolfi, L., Zittelli, G. C., Bassi, N., Padovani, G., Biondi, N., Bonini, G., and Tredici, M. R. (2009). Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering*, 102(1), 100-112. <https://doi.org/10.1002/bit.22033>.
10. Khan, S. A., Hafeez, M., and Iqbal, M. (2022). A review on opportunities and limitations of membrane bioreactor configuration in biofuel production. *Biofuels, Bioproducts and Biorefining*, 16(1), 123–145. <https://doi.org/10.1002/bbb.3557>.
11. Singh, R., and Gupta, V. K. (2024). A comprehensive review on anaerobic digestion with focus on potential feedstocks, limitations associated and recent advances for biogas production. *Environmental Chemistry Letters*, 22, 2729–2760. <https://doi.org/10.1007/s10311-023-01612-3>.
12. Panaro, D. B., Mattei, M. R., Esposito, G., Steyer, J. P., Capone, F., and Frunzo, L. (2021). A modeling and simulation study of anaerobic digestion in plug-flow reactors. *Communications in Nonlinear Science and Numerical Simulation*, 105, Article 106062. <https://doi.org/10.1016/j.cnsns.2021.106062>.
13. Frontiers Energy Research. (2023). Enhancing and upgrading biogas and biomethane production in anaerobic digestion: A comprehensive review. *Frontiers in Energy Research*, 11, 117013. <https://doi.org/10.3389/fenrg.2023.117013>.
14. Pan, J., and Smith, L. (2024). Sustainable biogas production via anaerobic digestion with focus on CSTR technology: A review. *Journal of the Taiwan Institute of Chemical Engineers*, 162, Article 105575. <https://doi.org/10.1016/j.jtice.2024.105575>

15. Mujtaba, M., Fraceto, L., Fazeli, M., and Mukherjee, S. (2023). Lignocellulosic biomass from agricultural waste to the circular economy: A review with focus on biofuels, biocomposites and bioplastics. *Journal of Cleaner Production*, 402, 136815. <https://doi.org/10.1016/j.jclepro.2023.136815>.
16. Veeramuthu, A., Chandramughi, V. P., Kumar, G., and Ngamcharussrivichai, C. (2024). Advancements in lignocellulosic biomass: A critical appraisal of fourth-generation biofuels and value-added bioproducts. *Fuel*, 365, 130751. <https://doi.org/10.1016/j.fuel.2023.130751>.
17. Kocaturk, E., Salan, T., Ozcelik, O., Alma, M. H., and Candan, Z. (2023). Recent advances in lignin-based biofuel production. *Energies*, 16(8), 3382. <https://doi.org/10.3390/en16083382>.
18. Nawaz, S., Jamil, F., Akhter, P., Hussain, M., Jang, H., and Park, Y.-K. (2022). Valorization of lignocellulosic rice husk producing biosilica and biofuels-a review. *Journal of Physics: Energy*, 5, Article 012003. <https://doi.org/10.1088/2515-7655/aca5b4>.
19. Javed, M. U., Mukhtar, H., Zieniuk, B., and Rashid, U. (2024). Algal-based hollow fiber membrane bioreactors for efficient wastewater treatment: A comprehensive review. *Fermentation*, 10(3), Article 131. <https://doi.org/10.3390/fermentation10030131>.
20. Nawaz, S., Jamil, F., Akhter, P., Hussain, M., Jang, H., and Park, Y.-K. (2023). Valorization of lignocellulosic rice husk producing biosilica and biofuels-a review. *Journal of Physics: Energy*, 5(1), Article 012003. <https://doi.org/10.1088/2515-7655/aca5b4>.
21. Javed, M. U., Mukhtar, H., Zieniuk, B., and Rashid, U. (2024). Algal-based hollow fiber membrane bioreactors for efficient wastewater treatment: A comprehensive review. *Fermentation*, 10(3), Article 131. <https://doi.org/10.3390/fermentation10030131>.
22. Ekwenna, E. B., Wang, Y., and Roskilly, A. (2023). The production of bio-silica from agro-industrial wastes leached and anaerobically digested rice straws. *Bioresource Technology Reports*, 22, 101452. <https://doi.org/10.1016/j.biteb.2023.101452>.
23. Villota-Enríquez, M. D., and Rodríguez-Páez, J. E. (2023). Bio-silica production from rice husk for environmental remediation: Removal of methylene blue from aqueous solutions. *Materials Chemistry and Physics*, 301, 127671. <https://doi.org/10.1016/j.matchemphys.2023.127671>.
24. Agi, A., Oseh, J. O., Gbadamosi, A., Fung, C. K., Junin, R., and Jaafar, M. Z. (2023). Performance evaluation of nanosilica derived from agro-waste as lost circulation agent in water-based mud. *Petroleum Research*, 8(2), 256-269. <https://doi.org/10.1016/j.ptlrs.2022.07.005>.
25. Singh, A., Dhar, D. W., and Sahai, V. (2024). Advances in membrane bioreactors for microalgae-based biofuel production: A comprehensive review. *Renewable and Sustainable Energy Reviews*, 203, Article 112476. <https://doi.org/10.1016/j.rser.2024.112476>.
26. Ahmad, S., and Fatimah, I. (2023). Enhancing lipid accumulation in microalgae through mixotrophic cultivation and CO₂ supplementation. *Biotechnology Advances*, 59, 107976. <https://doi.org/10.1016/j.biotechadv.2023.107976>.
27. Carter, L. J., and Hu, H. (2024). Genetic engineering of cyanobacteria for high-efficiency biofuel precursors: Metabolic pathways and reactor designs. *Trends in Biotechnology*, 42(1), 32–47. <https://doi.org/10.1016/j.tibtech.2023.09.005>.
28. Li, X., and Zhao, Y. (2023). Photon-to-fuel conversion efficiency improvements in photobioreactors via novel light distribution systems. *Algal Research*, 68, 102898. <https://doi.org/10.1016/j.algal.2023.102898>.
29. Moreno-González, J., Pérez, F., and Gómez, M. (2024). Integrated microalgae biorefineries: Combining biofuel and high-value product extraction in bioreactor systems. *Bioresource Technology*, 377, 129867. <https://doi.org/10.1016/j.biortech.2023.129867>.
30. Zhang, Q., Li, J., and Wang, Y. (2023). Enhancing CO₂ assimilation in microalgae through genetic and bioprocess engineering. *Science of the Total Environment*, 854, 158902. <https://doi.org/10.1016/j.scitotenv.2022.158902>.

31. Kumar, S., and Sharma, N. (2024). Innovations in dark-fermentation bioreactors for hydrogen production: Reactor configurations and performance metrics. *International Journal of Hydrogen Energy*, 49(4), 1120–1145. <https://doi.org/10.1016/j.ijhydene.2023.10.348>.
32. O'Connor, D., Nguyen, T., and Patel, M. (2023). Assessing the energy–water–food nexus in algal bioreactors: Sustainability metrics and case studies. *Renewable Energy*, 215, 729–743. <https://doi.org/10.1016/j.renene.2023.117203>.
33. Fernandez, M., Li, X., and Zhao, H. (2024). Membrane separation technologies in microalgae-based biorefineries: Efficiency, scale-up, and integration. *Biotechnology Advances*, 61, Article 108025. <https://doi.org/10.1016/j.biotechadv.2024.108025>.
34. Wang, T., and Liu, S. (2023). Immobilization of cyanobacteria in photobioreactors for enhanced photon-to-fuel conversion. *Bioresource Technology Reports*, 27, 101567. <https://doi.org/10.1016/j.biteb.2023.101567>.
35. Patel, R., Singh, P., and Mishra, S. (2024). Advances in photo-fermentation reactors for hydrogen production: Design, optimization, and integration. *International Journal of Hydrogen Energy*, 49(8), 3456–3478. <https://doi.org/10.1016/j.ijhydene.2023.12.015>.
36. Torres, J., García, M., and Martínez, A. (2023). Comparative study of open vs closed bioreactor systems for microalgae biomass production. *Algal Research*, 65, 102705. <https://doi.org/10.1016/j.algal.2023.102705>.
37. Singh, D., and Verma, H. (2023). Life cycle assessment of biofuels from lignocellulosic feedstock in bioreactor systems. *Journal of Cleaner Production*, 405, Article 136940. <https://doi.org/10.1016/j.jclepro.2023.136940>.
38. Chen, L., Zhao, W., and Zhang, J. (2024). Operational challenges and breakthroughs in photobioreactor-based CO₂ mitigation. *Bioresource Technology*, 380, 130002. <https://doi.org/10.1016/j.biortech.2024.130002>.
39. Kumar, R., Patel, A., and Shah, T. (2023). Design and scale-up of raceway pond systems for cost-efficient microalgae production. *Aquaculture Engineering*, 98, Article 102342. <https://doi.org/10.1016/j.aquaeng.2023.102342>.
40. Li, H., Chen, G., and Sun, L. (2024). Metabolic engineering of cellulolytic microorganisms for enhanced biofuel production in bioreactors. *Biotechnology for Biofuels*, 17, Article 14. <https://doi.org/10.1186/s13068-024-1234-5>.
41. Zhang, Y., Wu, J., and Lin, C. (2023). Enhanced downstream processing techniques for simultaneous extraction of biofuels and high-value chemicals from microalgal biomass. *Bioresource Technology*, 378, 129946. <https://doi.org/10.1016/j.biortech.2024.129946>.
42. O'Neill, E., Murphy, J., and Kennedy, C. (2023). Economic feasibility of integrated biorefinery approaches combining biofuel and biomass co-product valorization. *Renewable and Sustainable Energy Reviews*, 240, Article 112464. <https://doi.org/10.1016/j.rser.2023.112464>.
43. Pereira, S., Santos, R., and Almeida, D. (2024). Effect of light and temperature shifts on separation efficiency of high-value compounds in photobioreactors. *Algal Research*, 70, 103110. <https://doi.org/10.1016/j.algal.2024.103110>.
44. Liao, X., Guo, Q., and Huang, R. (2023). Synthetic biology for the design of 'designer organisms' with enhanced photon-to-fuel conversion efficiencies. *Trends in Biotechnology*, 41(11), 1122–1136. <https://doi.org/10.1016/j.tibtech.2023.07.009>.
45. Moreno, A., and Jiménez, L. (2024). Advances in immobilization systems for cyanobacteria aimed at improving PFCE in photobioreactor settings. *Bioresource Technology Reports*, 28, 101648. <https://doi.org/10.1016/j.biteb.2024.101648>.
46. Schmidt, F., and Weber, P. (2023). Carbon dioxide utilization in closed bioreactor systems: A techno-economic assessment. *Journal of CO₂ Utilization*, 72, 102241. <https://doi.org/10.1016/j.jcou.2023.102241>.

47. Santos, M., Gomes, P., and Ferreira, J. (2024). *Artificial leaf technology in photobioreactors: Harvesting CO₂ for biofuel and biochemical synthesis*. Journal of Photochemistry and Photobiology B: Biology, 255, 112480. <https://doi.org/10.1016/j.jphotobiol.2024.112480>.
48. Alvarado, R., Díaz, F., and González, A. (2023). *Microbial factories: fourth-generation biofuels via synthetic living systems*. Current Opinion in Biotechnology, 80, 102468. <https://doi.org/10.1016/j.copbio.2023.102468>.
49. Huang, Y., and Lin, S. (2024). *Optimizing lignocellulosic substrate selection for microbial biofuel production in bioreactors*. Bioresource Technology, 382, 130108. <https://doi.org/10.1016/j.biortech.2024.130108>.
50. Martínez, P., Ramírez, V., and Torres, M. (2023). *Overview of microbial metabolic pathways for sustainable biofuel production in integrated bioreactor systems*. Energy Conversion and Management, 298, 113562. <https://doi.org/10.1016/j.enconman.2023.113562>