

MICROBIAL ENGINEERING: A FUTURISTIC APPROACH FOR PRODUCTION OF SUSTAINABLE BIOFUELS

Abstract

The vitality of the economy is tightly connected to energy. As economies grow and populations increase the demand, for energy continues to rise. Finding alternatives to fuels has become a priority especially in addressing climate change ensuring energy security and working towards a more sustainable future. It is crucial to shift from fuels to options to tackle the challenges posed by increasing energy demand and environmental degradation. The need for alternatives becomes more important when considering the projected energy requirements in the coming decades with China and India playing a significant role in this increase. Where different microorganisms work together towards a common goal. To maximize the impact of biofuels, the range of raw materials used in next-generation biofuels should be expanded, and biofuel production should eventually use either substrates that result in a net increase in global CO₂ fixation or carbon that is released as greenhouse gases. As a result, the development of next-generation biofuels should take into consideration the emissions caused by the usage of land to grow and harvest biomass, the impact on biodiversity, and the increased manufacturing costs. Overall, the prospects of microbial engineering are promising, offering significant advancements in biofuel production and contributing to a greener and more sustainable energy future. In conclusion transitioning towards biofuels isn't just an option; it's a necessity, for building a resilient and environmentally conscious future of energy.

Keywords: Biofuels, Microorganisms.

Authors

Smriti Thakur

Post Graduation student
PG department of Biotechnology and
Microbial Biotechnology.
Sri Guru Gobind Singh College
Chandigarh, India.

Dr. Amit Joshi

Assistant professor
PG department of Biotechnology and
Microbial Biotechnology.
Sri Guru Gobind Singh College
Chandigarh, India.

Dr. Vikas Kushwaha

Assistant professor
PG Department of Biotechnology
and Microbial Biotechnology.
Sri Guru Gobind Singh College
Chandigarh, India.

I. INTRODUCTION

The global economy is actually powered by energy. Economic expansion, paired with expanding population, has resulted in a constant rise in worldwide energy demand. If current practices continue, the world will require over 60% more energy in 2030 than it does today, with China and India accounting for 45% of the total. Transportation is one of the fastest expanding industries, accounting for 27% of total primary energy use. Since 1870, fossil fuels have been the primary fuel source in the industry. The ongoing use of fossil fuels is non-sustainable as they are finite resources and their combustion will result in higher energy-related emissions of greenhouse gases (GHG), such as carbon dioxide (CO₂), sulfur dioxide (SO₂), and nitrogen oxides (NO_x) (Patil et al., 2008).

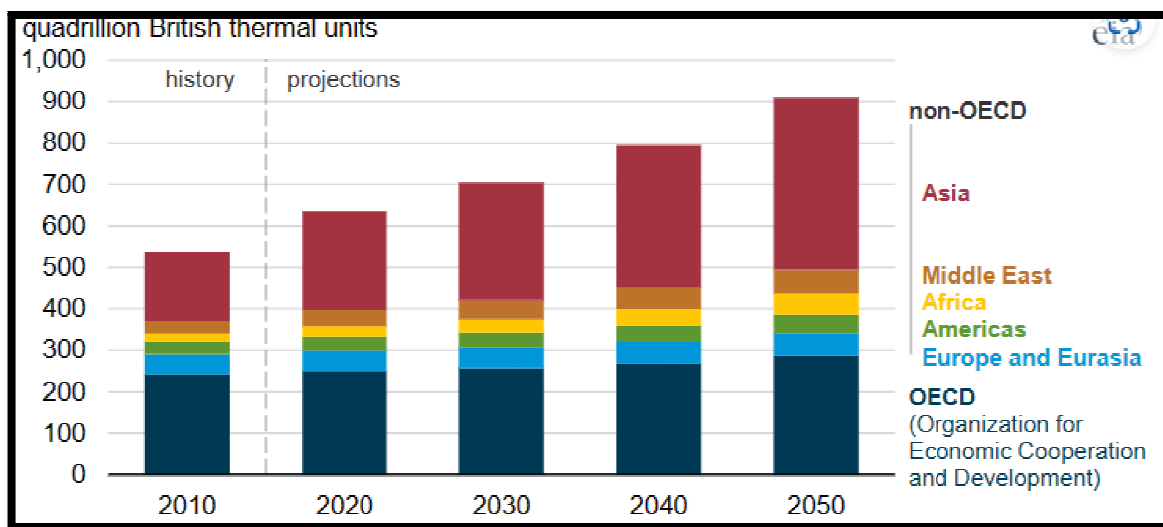


Figure 1: Regional consumption of primary energy in the world (2010-2050)) (EIA Projects Nearly 50% Increase in World Energy Usage by 2050, Led by Growth in Asia, n.d.).

Alternative transportation fuels are in high demand owing to concerns about climate change, the global petroleum supply and energy security (Lee et al., 2008). Biofuels such as ethanol, biodiesel, hydro processed vegetable oils and lipids, upgraded bio-pyrolisis oil (bio-oil), or biogas-derived biomethane derived from biomass and renewable resources such as lignocellulosic plants, starch or sugar crop plants, and the organic fraction of municipal or industrial waste. Ethanol, a common biofuel, is created from plants in the same way that ethanol is made for alcohol. Sugar beet, corn, and sugar cane are some of the main plants used in ethanol production due to their high sugar content, which can then be easily fermented by microbes such as the yeast (*Saccharomyces cerevisiae*). Biofuels have a much lower influence on climate change than fossil fuels. According to studies, biodiesel reduces hydrocarbon emissions by 75-90%, while using it instead of diesel reduces carbon monoxide and smog-causing particulate matter emissions by roughly 50%. It also completely eliminates all sulfur emissions (Davey, 2020).

To maximize the impact of biofuels, the range of raw materials used in next-generation biofuels should be expanded, and biofuel production should eventually use either substrates that result in a net increase in global CO₂ fixation or carbon that is released as

greenhouse gases. Furthermore, replacing fossil fuels with biofuels may not always result in lower CO₂ emissions, unless all ancillary emissions are taken into account. As a result, the development of next-generation biofuels should take into consideration the emissions caused by the usage of land to grow and harvest biomass, the impact on biodiversity, and the increased manufacturing costs(Liao et al., 2016).

To tackle these challenges, scientists are actively working on microbial engineering, where they modify the metabolic systems of microorganisms to boost output and efficiency while reducing the need for raw materials, plant size, capital investment, and operational expenses. Microbial engineering plays a vital role in biofuel production by modifying the genetic characteristics of microorganisms. This genetic modification enhances their ability to produce biofuels in a more efficient and sustainable manner. Engineered microbes can be fine-tuned to process a wide variety of feedstocks, making the biofuel production process more flexible and reducing reliance on limited resources. Moreover, microbial engineering allows the creation of consortia, where different microbes work together in harmony, resulting in higher biofuel yields and cost-effectiveness. Additionally, this approach enables the development of robust microbial strains that can thrive in harsh conditions and grow rapidly, thereby accelerating the speed and efficiency of biofuel manufacturing (Zhang et al., (2021). Overall, the prospects of microbial engineering are promising, offering significant advancements in biofuel production and contributing to a greener and more sustainable energy future.

Sustainable biofuels, commonly known as "second-generation" biofuels, originate from sources other than food, such as agricultural residue, algae, and inedible plant matter. In contrast to first-generation biofuels that utilize food crops like corn and sugarcane, second-generation biofuels address apprehensions related to food security and deforestation (Jeswani et al., 2020). In this chapter, we will explore a futuristic approach to the production of sustainable biofuels.

II. MICROBIAL DIVERSITY: A TREASURE TROVE FOR BIOFUEL PRODUCTION

The diversity of microorganisms offers a valuable reservoir for generating biofuels, owing to the inherent metabolic capacities of different microbial species. Forward-looking biofuel manufacturing takes advantage of the adaptability of microorganisms to effectively transform a broad spectrum of raw materials into biofuels. (Xiong et al., 2008). Recent developments in the field of bioresources have demonstrated that certain microorganism species, encompassing microalgae, yeast, and fungi, are enriched with substantial quantities of fatty acids. This discovery underscores the potential utilization of these microorganisms as viable candidates for sourcing biofuel production.

1. Engineered Microbes: Tailoring for Biofuel Synthesis: Microbes can be engineered to produce biofuels through metabolic pathways not naturally present in them. Genetic modifications enable microorganisms to efficiently convert sugars, lignocellulosic biomass, and even carbon dioxide into biofuels like ethanol, butanol, and fatty acid methyl esters (Weber et al., 2010).

In 2009, Huang and colleagues conducted a study that highlighted microbial oil production using waste rice straw. Through the cultivation of the microorganism *Trichosporon fermentans*, microbial oil was synthesized from sulphuric acid-treated rice straw hydrolysate (SARSH). Notably, *T. fermentans* exhibited the capability to metabolize various sugars, including mannose, galactose, and cellobiose, found in hydrolysates from different natural lignocellulosic materials used as carbon sources. This microorganism demonstrated proficient growth on rice straw hydrolysate, effectively accumulating lipid content within its cellular biomass with a notably high yield. As such, *T. fermentans* emerged as a promising candidate for microbial oil production. (Singh & Nigam, 2014). Additionally, Zhu and co-researchers focused on generating microbial biofuel from waste molasses. Their findings revealed that lipids generated within the microbial biomass could be employed for biodiesel production. The introduction of different sugars into the pretreated molasses led to a substantial augmentation in lipid accumulation within microbial cells, resulting in lipid content exceeding 50% of the cell mass. The composition of microbial lipid, akin to vegetable oils, predominantly encompassed palmitic, stearic, oleic, and linoleic acids, with unsaturated fatty acids constituting approximately 64% of the total fatty acid content. The effective growth of yeast on pretreated lignocellulosic biomass markedly bolstered lipid accumulation, providing a promising avenue for economically and environmentally viable microbial oil production from agricultural residues (Singh & Nigam, 2014).

- 2. Algae: The Microbial Biofuel Producers:** Algae, recognized as some of the most ancient life forms, inhabit diverse ecosystems across the Earth, encompassing a wide array of species that thrive in various environmental conditions. These organisms belong to the category of primitive plants (thallophytes), characterized by the absence of roots, stems, and leaves. They also lack a protective layer of cells around their reproductive cells and primarily utilize chlorophyll a for photosynthesis. In their natural habitats, phototrophic algae capture sunlight and absorb carbon dioxide from the atmosphere, along with nutrients from aquatic environments. Microalgae possess the remarkable ability to rapidly generate substantial quantities of lipids, proteins, and carbohydrates within short timeframes. (Patil et al., 2008). These products hold the potential for conversion into biofuels as well as valuable co-products. Nonetheless, the synthesis of lipids, proteins, and carbohydrates might face limitations imposed by available sunlight due to diurnal patterns and seasonal fluctuations. Consequently, the feasibility of large-scale commercial production could be constrained to regions characterized by abundant solar radiation.

Producing microalgal biodiesel necessitates significant amounts of algal biomass, with the potential to yield over 250 times the oil output per acre compared to soybeans. Likewise, algae has the capacity to generate 7 to 31 times more biodiesel compared to palm oil. There's a notion that biodiesel derived from algae could potentially serve as a substitute for gasoline in automotive fuel applications (Amer et al., 2018).

The majority of algal species rely on light as obligate phototrophs for their growth, making light a vital requirement. Numerous cultivation methods aimed at producing microalgal biomass have been formulated by both researchers and commercial practitioners. Phototropic microalgae are commonly cultivated in open ponds and photobioreactors. Photobioreactors present a confined cultivation environment, shielding

the culture from direct contamination, minimizing the risk of unwanted microorganism intrusion, and enabling temperature control, all while facilitating enhanced carbon dioxide fixation through the introduction of CO₂ into the culture medium. However, the associated infrastructure expenses render this technology comparatively costly compared to open ponds (Singh & Nigam, 2014). The most optimal approach for biomass production should leverage the freely available sunlight. Various designs of photobioreactors have been devised, yet the tubular photobioreactor configuration appears to be the most effective for generating the required scale of algal biomass suitable for biofuel manufacturing. Enclosed, controlled indoor algal photobioreactors illuminated by artificial light have already proven to be financially viable for specialized high-value products like pharmaceuticals. This technology can be integrated with biodiesel production to reduce overall costs.

III. METABOLIC ENGINEERING: REWITING MICROBIAL BLUEPRINTS FOR BIOFUEL SYNTHESIS

Microorganisms have a dual role in the production of a diverse array of biofuels. They contribute both directly and indirectly to this process. In the direct realm, heterotrophic microorganisms have emerged as key players in the industrial synthesis of biofuels such as biogas and fuel alcohols by converting organic materials. Conversely, photosynthetic microorganisms play a distinct role by converting inorganic carbon and water into promising fuel derivatives like fuel alcohols and biohydrogen. Additionally, they contribute to the production of fuel precursors like biomass, starch, and lipids. (Ramsey, 2013). Presently, the utilization of microbial processes for the commercial production of biofuels is somewhat limited; however, this landscape is anticipated to evolve markedly as advances in microbial metabolic engineering continue to bolster production capacities. Previously complex procedures, involving multiple stages of feedstock pretreatment followed by fuel conversion, are being streamlined into unified microbial processes through the manipulation of metabolic pathways in engineered microorganisms. Moreover, microorganisms are being genetically modified to facilitate the generation of fuels from feedstock that was previously unusable to them (Keasling et al., 2021).

1. Synthetic Biology: Building Customized Pathways: The emergence of synthetic biology marks a novel phase in the field of microorganism metabolic engineering. This phase is characterized by the construction of biochemical pathways for the synthesis of biofuels and natural products, accomplished by assembling these pathways from components originating from diverse sources. The successful execution of such endeavors is highly contingent on the advancement and adept application of computational tools (Medema et al., 2012). Synthetic biology operates by crafting artificial biological systems, achieved through the amalgamation of engineering strategies and biotechnological tools. This process relies on insights derived from genetic, metabolic, and regulatory experiments. In simpler terms, synthetic biology constitutes a biological discipline centered around the reconstruction or modification of metabolic pathways through the integration of genetic modules, often referred to as "biological circuits".(Jagadevan et al., 2018)

A considerable portion of early commercial applications in synthetic biology revolves around the manipulation of metabolic pathways to replicate naturally occurring compounds. Through the utilization of synthetic biology methods, genetically engineered agricultural crops are being developed to function as feedstocks for the production of biofuels. (Brooks & Alper, 2021)

- 2. Substrate for Biofuel Production:** In the pursuit of mitigating or preventing secondary emissions, the raw components required for sustainable biofuel generation ought to originate from atmospheric CO₂, achieved through a net augmentation of global carbon fixation, or from carbon emissions that presently function as greenhouse gases without optimal utilization. These resources need to be present in substantial quantities to effectively supplant a substantial segment of the existing fossil fuels consumption. While we do not delve into the sustainable derivation of these raw materials in this context, our focus remains directed towards elucidating the methods by which they can be transformed into biofuels. (Liao et al., 2016).

Lignocellulosic biomass, denoting non-edible photosynthetic residual materials, presents a substantial potential and expansive avenue for substituting fossil fuels and diminishing energy reserves in the industrial creation of petrochemicals and biofuels. When it comes to starch biomass, a direct saccharification step is necessary, whereas in the case of sucrose-based lignocellulosic materials, this step becomes unnecessary since sugars are already present, thus simplifying the process. Biomass, acting as a replacement for fossil fuels, particularly in the production of ethanol, includes resources like sugarcane, corn, jatropha, and microalgae, all of which exert a reduced ecological impact and are renewable (Sadia et al., 2020). For instance, substrates sourced from forest wood and crop residues are harnessed for bioethanol fermentation, giving rise to what is termed second-generation biofuels. A subsequent tier, known as third-generation biofuels, leverages microalgae as the primary substrate, while fourth-generation biofuels employ modified microalgae generated through postgenomic technologies. As for biofuels originating directly from cellulose, hemicellulose, and lignin, they are classified under the second-generation category. The preference for lignocellulosic biomass over food-based crops stems from its ability to avoid disrupting the food chain and compromising energy security. Prominent substrates like corn stover, wheat straw, rice husk, and sugarcane bagasse have consistently been regarded as reliable resources for biofuel production.

- 3. Pathway Engineering for Advanced Biofuels:** The process of biofuel production centres on refining metabolic pathways to achieve optimal productivity and output. Metabolic engineering and synthetic biology play pivotal roles in enabling the advancement of novel technologies. However, challenges arise due to the potential chemical toxicity of substrates, intermediates, and end products. These issues can disrupt metabolic processes and harm the cellular structure. As a result, it becomes essential to formulate engineering approaches aimed at enhancing the resilience of microorganisms to the stresses mentioned above (Khalidi et al., 2016)
 - **Ethanol Pathway:** Within microorganisms, the production of ethanol can occur through pyruvate decarboxylation, either with or without the involvement of acetyl-CoA. For instance, *S. cerevisiae* and *Z. mobilis*, which are recognized as two of the most proficient ethanol producers, engage in the direct decarboxylation of pyruvate,

followed by its conversion into ethanol through reduction, bypassing the utilization of acetyl-CoA in the process (Liao et al., 2016). In contrast, a majority of other microorganisms, including *Escherichia coli* and *Clostridium spp.*, opt for the incorporation of CoA to activate the acyl group during pyruvate decarboxylation before the subsequent reduction into ethanol. This dissimilarity underscores the notion that the direct decarboxylation route is a more efficient pathway, consequently leading to the integration of this direct pathway into various other organisms through engineering efforts.

- **Keto Acid Pathway:** In a manner similar to the pyruvate decarboxylation process for ethanol production observed in *S. cerevisiae* and *Z. mobilis*, the decarboxylation of longer-chain keto acids offers the potential for generating longer-chain alcohols (Liao et al., 2016). This strategy involves extending the carbon chain at the level of 2 keto acids, which play a role in the biosynthesis of branched chain amino acids. The elongation of the keto acid chain initiates through enzymes like acetohydroxyacid synthase within the valine biosynthesis pathway, or isopropylmalate synthase in the context of the leucine biosynthesis pathway. Subsequently, a series of steps including isomerization, reduction (or oxidation), and dehydration contribute to the regeneration of the 2 keto acid functional group.
- **Isoprenoid Pathway:** Isoprenoid monomers can be derived either from acetyl-CoA through the mevalonate pathway or from pyruvate and glyceraldehyde-3-phosphate (G3P) via the deoxyxylulose phosphate (DXP), also known as the methylerythritol phosphate (MEP) pathway. These monomers, composed of five carbons (such as IPP and DMAPP), possess the potential to polymerize into chains spanning from a single unit to over a dozen. The feasibility of generating fuel molecules hinges on the augmentation of precursor biosynthesis and the optimization of multiple subsequent stages (Swapnil et al., 2008). Isoprenoids, exemplified by phytoene (C40), function as intermediates in the synthesis of larger isoprenoid compounds, and they could potentially serve as suitable candidates for biocrude production. Notably, both *E. coli* and *S. cerevisiae* have been effectively engineered to produce carotenoids based on phytoene. Furthermore, aside from biocrude generation, recent research indicates the isoprenoid pathway's potential utility in the production of proposed gasoline additives like isopentanol and isoamyl acetate.
- **Fatty Acid Biosynthesis:** Fatty acids stand as fundamental metabolites, their synthesis orchestrated by intricate and indispensable biosynthetic machinery. Fatty acid synthases resemble a sequential assembly line, wherein an acyl carrier protein transports the evolving fatty acid to the requisite enzymatic domains for modification (Beld et al., 2015).

The biosynthesis of fatty acids employs chemistry akin to β -oxidation, the process for fatty acid degradation, although it employs ACP for the activation of the acyl group (Liao et al., 2016). Ordinarily, the initiation of fatty acid biosynthesis originates from acetyl-CoA, followed by carboxylation to generate malonyl-CoA units. These building blocks are subsequently merged and reduced in an iterative manner until the fatty acid chain reaches maturity, ready for cellular utilization (Beld et al., 2015).

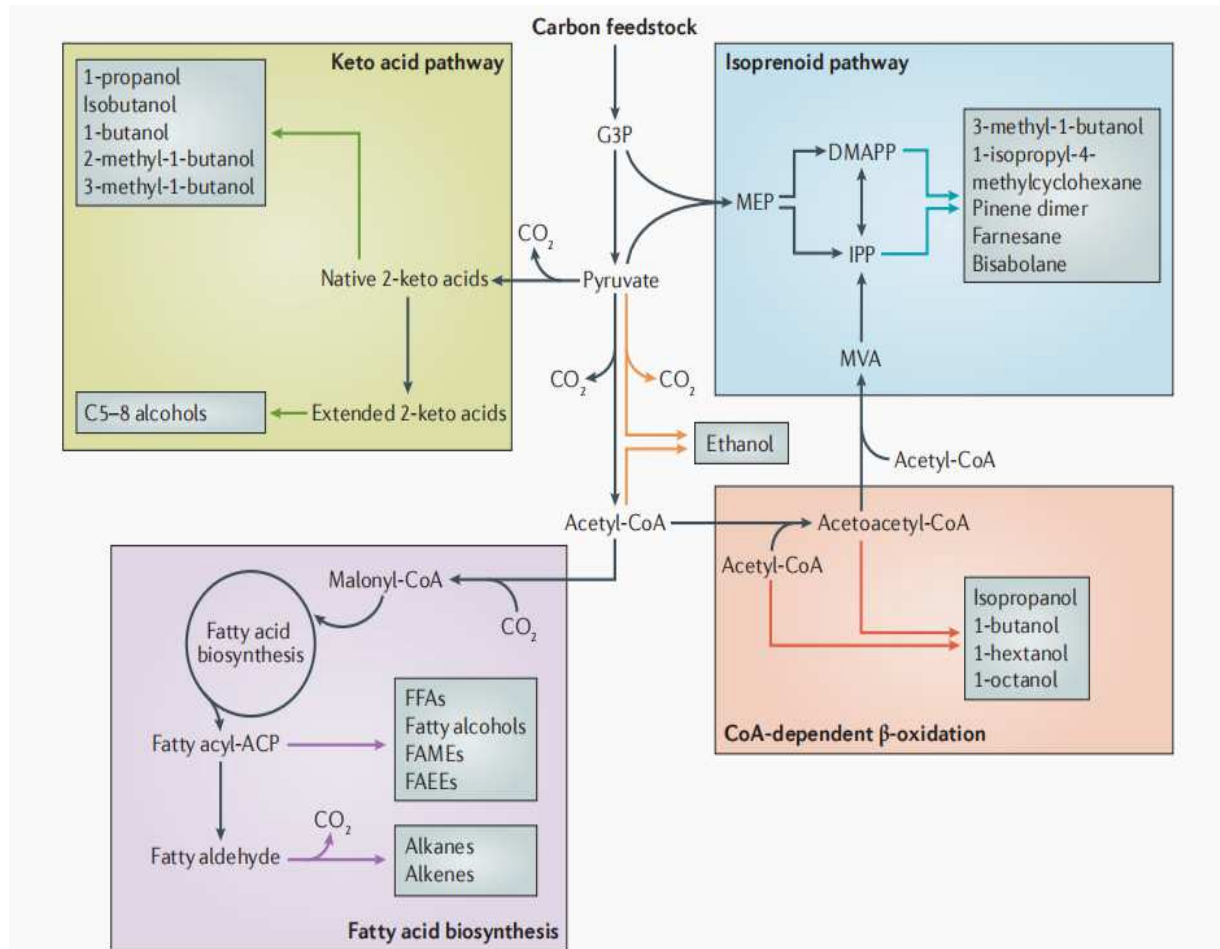


Figure 2: Biosynthetic pathways of biofuels. Ethanol can be derived from either pyruvate or acetyl CoA with acetaldehyde serving as an intermediate. The keto acid pathway allows for the production of both straight chain alcohols utilizing elements of amino acid biosynthesis pathways to elongate the keto acid chain. This is followed by decarboxylation and reduction of the keto acid to how pyruvate is converted into ethanol. Fatty acid synthesis involves extending acyl acyl carrier proteins (ACPs) using malonyl CoA as a precursor. Fatty acyl ACPs can be converted into fatty acids (FFAs) through the action of acyl ACP thioesterase. FFAs have the potential to form esters like fatty acid methyl esters (FAMES) or fatty acid ethyl esters (FAEEs) be reduced to fatty alcohols or undergo reduction to form fatty aldehydes followed by decarbonylation, into alkanes and alkenes. The CoA dependent pathway employs β oxidation chemistry for producing alcohols or decarboxylation of the precursor acetoacetyl CoA for generating isopropanol. Isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) which serve as precursors, for isoprenoid biosynthesis can be synthesized via either the mevalonate (MVA) pathway or methylerythritol 4 phosphate (MEP) pathway. G3P, also known as glyceraldehyde-3-phosphate (Liao et al., 2016).

- CoA-dependent Reverse β -oxidation:** The CoA-dependent reverse β -oxidation pathway mirrors the chemical reactions of β -oxidation, albeit in the opposite direction for fatty acid degradation. Unlike the process of fatty acid biosynthesis, this pathway employs CoA rather than acyl carrier protein (ACP) for the activation of the acyl

group. This pathway finds application in the generation of primary alcohols, such as 1-butanol, 1-hexanol, and secondary alcohol like isopropanol, in various species of *Clostridium*.

In this particular pathway, two acetyl-CoA molecules undergo condensation to yield acetoacetyl-CoA, which subsequently undergoes reverse β -oxidation to produce 1-butyryl-CoA. This acyl-CoA can then be subjected to reduction to yield 1-butanol, or it can proceed through further cycles to extend the chain length by two carbon atoms in each iteration, leading to the eventual production of 1-hexanol, 1-octanol, or even longer-chain alcohols. Moreover, acetoacetyl-CoA can be transformed into isopropanol after undergoing decarboxylation (Liao et al., 2016).

III. CHALLENGES AND FUTURE DIRECTIONS

In light of the significant environmental apprehensions arising from the extensive development of large-scale biofuels – encompassing issues like the "food versus fuel" debate, deforestation, water pollution, and water scarcity, among others – as well as the socio-economic considerations, the past decade has witnessed a mounting acknowledgment of the imperative to establish sustainability criteria and certification standards for both the production and trade of biofuels. Lewandowski and Faaij conducted an assessment of the existing environmental certification systems to determine their relevance to the expanding bioenergy trade (Solomon, 2010).

Given the substantial quantity of biofuels required and their relatively low unit price, the efficiency of biofuel production becomes a paramount concern. Every conceivable avenue to enhance yield, titre, and productivity must be explored. The initiation of most metabolic engineering strategies involves the amplification of the desired pathway genes, succeeded by the elimination of competing pathways that siphon off precursors, products, or cofactors. Introducing novel pathways for precursor supply has also demonstrated its efficacy (Liao et al., 2016).

IV. CONCLUSION: A BRIGHT FUTURE FOR SUSTAINABLE

The global economy's vitality is intrinsically linked to energy, and as economies expand and populations grow, the demand for energy continues to rise. The increasing demand for energy is particularly evident in the transportation sector, which consumes a significant portion of total primary energy. Fossil fuels, the traditional energy source, have been the backbone of the industry since the 19th century. However, their continued use is not sustainable due to their finite nature and the adverse environmental impacts associated with their combustion. The pressing need for alternatives to fossil fuels has led to the exploration of biofuels, particularly in the context of mitigating climate change, ensuring energy security, and advancing towards a more sustainable energy future.

The transition from fossil fuels to sustainable alternatives is imperative to address the challenges posed by rising energy demand and environmental degradation. The urgency to find viable alternatives becomes even more pronounced when considering the projected energy requirements for the coming decades, with China and India accounting for a substantial portion of the increase. Transportation, a pivotal industry in global commerce, is

evolving rapidly and consequently accelerating energy consumption rates. This, in turn, drives the pursuit of cleaner, more sustainable energy sources. The significance of alternative transportation fuels, especially biofuels, has garnered attention due to their potential to mitigate climate change, diversify energy sources, and reduce reliance on petroleum. Biofuels, derived from renewable biomass sources, offer a promising pathway to reducing greenhouse gas emissions and enhancing energy security. The emergence of next-generation biofuels, derived from non-food sources like agricultural residue and algae, addresses concerns about food security and environmental sustainability. Such biofuels hold the potential to reshape the energy landscape, but challenges must be overcome to ensure their viability and positive impact.

Microbial engineering stands as a futuristic approach at the forefront of biofuel production. Through genetic modification and synthetic biology, microorganisms are being tailored to become efficient biofuel factories. Microbial engineering unlocks the potential of diverse microorganisms, ranging from bacteria to algae, to convert a wide spectrum of feedstocks into valuable biofuels. By optimizing metabolic pathways and enhancing microbial capabilities, engineered strains can efficiently produce biofuels such as ethanol, butanol, and bio-oil. Synthetic biology, a multidisciplinary field that combines biology and engineering, empowers scientists to design and engineer microorganisms for specific functions. This approach allows the creation of customized microbial platforms for biofuel synthesis. Microorganisms engineered through synthetic biology exhibit enhanced efficiency, resilience, and adaptability, making them ideal candidates for large-scale biofuel production.

The utilization of microbial consortia, where different microorganisms collaborate to achieve a common goal, represents a promising avenue to enhance biofuel yields and reduce production costs. Consortia take advantage of the strengths of individual microbes, resulting in a synergistic effect that optimizes biofuel production. Despite the promising prospects of microbial engineering, challenges remain on the path to sustainable biofuel production. Strain stability, scalability, and the environmental impact of land use must be addressed to ensure the long-term viability of biofuel production processes. Moreover, regulatory frameworks, public perception, and ethical considerations surrounding genetic modification necessitate careful evaluation and transparent communication.

In conclusion, the transition towards sustainable biofuels is not merely an option but a necessity to ensure a resilient and environmentally conscious energy future. Microbial engineering, with its potential to revolutionize biofuel production through genetic modification, synthetic biology, and consortia approaches, offers a path to a cleaner, greener energy landscape. As technology advances, interdisciplinary collaborations continue, and global efforts are united, the dream of a sustainable and vibrant energy future powered by biofuels becomes increasingly achievable. By harnessing the power of microorganisms and the principles of synthetic biology, the world can pave the way towards a more sustainable and harmonious coexistence with our planet.

REFERENCES

- [1] Amer, M., Saad, A., & Ismail, N. K. (2019). Biofuels from microorganisms: from current status to practical implementation.
- [2] Beld, J., Lee, D. J., & Burkart, M. D. (2015). Fatty acid biosynthesis revisited: structure elucidation and metabolic engineering. *Molecular BioSystems*, 11(1), 38-59.
- [3] Brooks, S. M., & Alper, H. S. (2021). Applications, challenges, and needs for employing synthetic biology beyond the lab. *Nature Communications*, 12(1), 1390.
- [4] *EIA projects nearly 50% increase in world energy usage by 2050, led by growth in Asia*. (n.d.). <https://www.eia.gov/todayinenergy/detail.php?id=42342>
- [5] Davey, R. (2020, 9 8). *The Use of Microbes in Biofuel Production*. (New medical life sciences) Retrieved from <https://www.news-medical.net/life-sciences/The-Use-of-Microbes-in-Biofuel-Production>.
- [6] Jagadevan, S., Banerjee, A., Banerjee, C., Guria, C., Tiwari, R., Baweja, M., & Shukla, P. (2018). Recent developments in synthetic biology and metabolic engineering in microalgae towards biofuel production. *Biotechnology for biofuels*, 11, 1-21.
- [7] Jeswani, H. K., Chilvers, A., & Azapagic, A. (2020). Environmental sustainability of biofuels: a review. *Proceedings of the Royal Society A*, 476(2243), 20200351.
- [8] Keasling, J., Garcia Martin, H., Lee, T. S., Mukhopadhyay, A., Singer, S. W., & Sundstrom, E. (2021). Microbial production of advanced biofuels. *Nature Reviews Microbiology*, 19(11), 701-715.
- [9] Khalidi, O., Guleria, S., & Koffas, M. A. (2016). Pathway and strain design for biofuels production. In *Biotechnology for Biofuel Production and Optimization* (pp. 97-116). Elsevier.
- [10] Lee, S. K., Chou, H., Ham, T. S., Lee, T. S., & Keasling, J. D. (2008). Metabolic engineering of microorganisms for biofuels production: from bugs to synthetic biology to fuels. *Current opinion in biotechnology*, 19(6), 556-563.
- [11] Liao, J. C., Mi, L., Pontrelli, S., & Luo, S. (2016). Fuelling the future: microbial engineering for the production of sustainable biofuels. *Nature Reviews Microbiology*, 14(5), 288-304.
- [12] Medema, M. H., Van Raaphorst, R., Takano, E., & Breitling, R. (2012). Computational tools for the synthetic design of biochemical pathways. *Nature Reviews Microbiology*, 10(3), 191-202.
- [13] Patil, V., Tran, K. Q., & Giselrød, H. R. (2008). Towards sustainable production of biofuels from microalgae. *International journal of molecular sciences*, 9(7), 1188-1195.
- [14] Ramsey, M. T. (2013). Microbes in Biofuel production: A review. *ResearchGate*. https://www.researchgate.net/publication/253327774_Microbes_in_Biofuel_Production_A_Review
- [15] Sadia, S., Bakhtawar, J., Irfan, M., Shakir, H. A., Khan, M., & Ali, S. (2021). Role of substrate to improve biomass to biofuel production technologies. *Bioprocessing for Biofuel Production: Strategies to Improve Process Parameters*, 127-156.
- [16] Singh, A., & Nigam, P. S. (2018). Microbial biofuels production. In *Microbial Biotechnology* (pp. 170-183). CRC Press.
- [17] Solomon, B. D. (2010). Biofuels and sustainability. *Annals of the New York Academy of Sciences*, 1185(1), 119-134.
- [18] Swapnil, C., Aindrila, M., Howard, C., Taek Soon, L., & Eric, S. (2008). Biofuel alternatives to ethanol: pumping the microbial well. *Trends in Biotechnology*, 26(7).
- [19] Weber, C., Farwick, A., Benisch, F., Brat, D., Dietz, H., Subtil, T., & Boles, E. (2010). Trends and challenges in the microbial production of lignocellulosic bioalcohol fuels. *Applied microbiology and biotechnology*, 87, 1303-1315.
- [20] Xiong, W., Li, X., Xiang, J., & Wu, Q. (2008). High-density fermentation of microalga *Chlorella protothecoides* in bioreactor for microbio-diesel production. *Applied microbiology and biotechnology*, 78, 29-36.
- [21] Zhang, S., & Liu, Z. (2021). Advances in the biological fixation of carbon dioxide by microalgae. *Journal of Chemical Technology & Biotechnology*, 96(6), 1475-1495.