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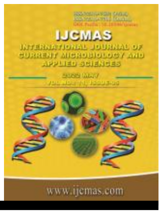

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Challenges and Opportunities in Bioprospecting for Sustainable Biofuel Production: Current Status and Future Perspectives

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ABSTRACT

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Increasing global energy demand and environmental concerns associated with petroleum have raised interest in biofuels to reduce dependency on crude oil and promote carbon-neutral energy generation. The information available suggests that biomass can become a sustainable and significant contributor to current energy demands if research and development in the field of thermochemical transformation for various biomass types are encouraged. The primary products of biofuel may be in a gas, liquid, or solid form. These products can be further converted by biochemical, physical, and thermochemical methods. The first generation of biofuels is ethanol derived from food crops rich in starch or biodiesel made from waste animal fats such as cooking grease. The second generation is bioethanol derived from non-food cellulosic biomass and biodiesel taken from oil-rich plant seeds, such as soybeans or jatropha. The third generation of biofuels is made from cyanobacteria, microalgae, and other microbes, and it is the most promising approach to meeting the world's energy demands. The era of biofuel production desires the ability to conclude formal incorporation of functional genomics metabolomics with transcriptomics will undoubtedly support the discovery. This review focuses on the production of biofuel through molecular marker technology, next-generation technology, and biochemical process.

Introduction

Energy is the most crucial resource for human development and economic growth. Throughout history, energy has been the major driver of the progression of human civilization. With the advancement of technology and a rapidly evolving society, along with an increasing population, the global energy demand is rising at a fast pace. This energy demand is more pronounced in developing countries with rapid urbanization and an increasing population (Asghar, 2008). At present, India's energy consumption is higher and is expected to increase in the future, owing to its increasing population, modernization, and economic growth (Thambi *et al.*, 2018; Asif *et al.*, 2007). Currently, India's energy requirements are met through conventional sources, which lead to higher greenhouse gas emissions, contributing to global warming. Moreover, the depletion of global fossil-fuel reserves, increasing environmental pollution, political conflicts, and an increase in fuel prices raise serious concerns about the continuation of conventional sources of energy for meeting energy requirements (OECD, 2012; Asif *et al.*, 2007, Panwar *et al.*, 2011). This alarming condition provides a sign for shifting toward alternatives. Biofuels seem to be a promising alternative to fossil fuels. Since these are derived from biomass and are biodegradable, renewable, and do not pose a threat to the environment, they arise as the best possible alternative (Godbole *et al.*, 2006).

Biomass generally includes agricultural residues and wastes, animal wastes, municipal wastes, crops, aquatic plants and algae, wastes from food processing, wood and logging residues, grass, etc (Demirbas, 2009). Biofuel production involves the adaptation of biomass into useful energy through various technologies and processes that involve physical, thermochemical, and biochemical processes (Kishore, 2003). The biomass used for producing biofuels includes a variety of feedstocks such as food crops, agricultural wastes, and microalgae. Unlike the other feedstocks available, agricultural wastes and remains, including animal wastes, make up the best source of biomass for biofuel production, as they do not require additional land for their growth and do not contribute additional greenhouse gas emissions. Utilizing these for the manufacture of energy would avoid methane emissions from landfills and slurry (Blaschek *et al.*, 2010). Moreover, the problem associated with the management of agricultural waste seems to have a solution, with these being used as feedstock for biofuel production. Agricultural wastes pose a greater threat to the environment and are a major contributor to air pollution, and in an agrarian country like India, this problem is more pronounced (Singh *et al.*, 2018). The disposal of crop residues and livestock waste has become a major problem for farmers. The management of this waste has an adverse economic and environmental impact associated with it. The practice of crop residue burning for the management of agricultural crop residues has a significant contribution to the problem of air pollution (Zhang *et al.*, 2011). This harms the health of human beings and livestock (Kumar *et al.*, 2016; Singh *et al.*, 2018). The production of biofuels from agricultural wastes provides the most suitable alternative for the management of agricultural wastes. This helps to eliminate the problem of environmental pollution due to agricultural residues and provides a sustainable energy supply in a cost-effective manner (Demirbas, 2009). Apart from conventional methods used for biofuel production, today's use of bioinformatics technologies such as ZFNs, TALENs, and CRISPR/Cas9 systems is being used to design and operate fresh strains of microorganisms with improved capacity for biofuel production from the genome level (Adegboye *et al.*, 2021).

Growing Bioenergy demand (global and domestic scenario)

Energy is the most important precursor for continuous human development. It is one of the most essential needs of human beings. We can say that energy drives human life. The achievements of every civilization that has ever existed on earth are the outcomes of their ability to harness various forms of energy efficiently to extend human capabilities. As per the data available, the global energy demand is increasing rapidly due to rapidly growing population, modernization, and urbanization. There are estimates that in the coming years the global energy demand will grow sharply Asif *et al.,* (2007); Renewable Energy World editorial (2003). For eradicating poverty, raising the living standards of the people worldwide, satisfying the innate aspirations of people for a better life, sustaining the human explorations and scientific adventures the supply of affordable and easily available energy is essential. Various currently employed energy systems seem to be not able to successfully deal with the future energy requirements (Asif *et al.,* 2007). Throughout the world, the current energy supply is dependent on fossil fuels and nuclear sources. Fossil fuel reserves are depleting and near to their exhaustion. The issues related to the economics and the safety of nuclear energy is still debatable and controversial. Moreover, the environmental concerns and threats related to human health and quality of life are connected with the production and consumption of energy through these conventional means and these could not be undermined (Abbott, 2009; Asif *et al.,* 2007).

Since there are increasing environmental concerns and there is a growing energy demand, and with the inability of conventional sources to satisfy the future energy demands, it is therefore expected that a conservational approach towards energy and alternative energy sources is expected. Newer energy systems that are renewable are being consistently and intensively investigated and are being developed (Ellabban *et al.,* 2014). The use of renewable energy is increasing with the emergence of problems like shortages of coal, natural gas, and other fossil fuels. Tremendous utilization of resources and overpopulation is the root cause of the need to look for alternative energy sources rather than conventional ones. The power sector saw a rise in profits, with the renewable energy sector witnessing 4% growth in the year 2018. With photovoltaic cells, hydropower, and wind energy generation contributing to one-third of this growth, the major portion was piled up by bioenergy generation during this period. Renewable energy has increased its capacity and is thus responsible for 40% of the world's electricity generation growth, which accounts for now over 20% of global power output.

The key players in renewable electricity generation are China (~40%), the European continent (~25%) followed by US and India (~13% combined generation) and then the rest of the world accounts for 22%. Many other countries are now realizing the importance of the technologies related to the production of renewable fuels.

This is evident by the fact that biofuel manufacturing has increased by 6% in the year 2018. Brazil's ethanol production rate went up by 12% due to the deterioration of international sugar prices and also because of the increased demand for conventional oils at the time of the global price surge. The production of ethanol in the US kept on rising in the year 2018, supported by abundant corn crop cultivation around the year and efficient utilization of domestic ethanol scaling up facilities. China witnessed a record growth of 24% in ethanol processing. The country's energy consumption policy triggered the implementation of the nationwide consumption of 10% ethanol blended with gasoline.

India has great potential for bioenergy generation, facilitated by a large supply of feedstock as raw material sources and moderately higher oil taxes eventually resulting in elevated oil commodity rates. The research focused on novel renewable resources state the enormous potency of Bioenergy and its constituent by-products including its traditional uses in developing countries, which is an abundant renewable source. While a decline in traditional uses would improve global health, modern use of bioenergy in industry and buildings holds great potential (Global energy and CO2 status report, 2018).

Biofuels: Classification, sources and production

Biofuels are classified according to the biomass feedstock source from which they have been produced. At present, four generations of biofuels have been introduced, namely, first-generation biofuels, second-generation biofuels, third-generation biofuels, and fourth-generation biofuels, generally abbreviated as 1G, 2G, 3G, and 4G, respectively (Moravvej, 2019). However, only first and second-generation biofuels have been commercially produced to date (Figure 1) (Abdullah, 2019).

First Generation Biofuels

First-generation biofuels are those in which the feedstock used is edible crops and whose sugars can be fermented by the microbial action on them (Albers, 2016; Akkari, 2018 and Kim, 2020).

First-generation biofuels are mainly produced from sugar, starch, and oil-based crops (Adbdullah, 2019). First-generation biofuels like ethanol and biodiesel are utilized mainly for transport (Peralta-Yahya, 2012).

The first generation of biofuels, unlike fossil-based fuels, can reduce GHG emissions (Moravvej, 2019). First-generation biofuels do not have any regulatory concerns, and production is economical (Abdullah, 2019). Even though first-generation biofuels are positioned to be a substitute for fossil-based fuels, they have their own set of limitations.

The first generation will be unable to meet global demand in a sustainable manner (Villareal, 2020). Its use can amplify the burden on agricultural lands. So, the land use requirement and its direct effect on food prices have made it a controversial product (Albers, 2016; Akkari, 2018). Biodiesel produced from oilseed plants such as soybean has limitations in oxidative stability and low oil yield (Thangavelu, 2020). On encountering these limitations, researchers have introduced more generations of biofuels, which have been discussed further.

Second Generation Biofuels

Second-generation biofuels' feedstock includes biomass or cellulosic feedstock that is the co-products, produced alongside crops, forestry, or waste streams like municipal waste or livestock manure. These don't clash with the food demands, like that of first-generation biofuels (Albers, 2016; Milessi, 2020). Lignocellulosic feedstocks are rich in fermentable sugars, majorly glucose and xylose, which can be obtained from the cellulose and hemicellulose content of the plant. Second-generation biofuels evade the limitations of first-generation biofuels, as well as climate change mitigation and rural economic development, some of their projected benefits (Lynd, 2017). Second-generation biofuels can cause better GHG reductions than their first-generation counterparts (Peralta-Yahya, 2012; Akkari, 2018; Milessi, 2020). The main sources of the second generation of biofuels, the cellulosic feedstocks, are the cheapest and are considered to have the greatest potential for mitigating climate change (Lynd, 2017). Cellulosic biomass exists in the form of wood, grass, and different types of waste that are not edible. Lignocellulose is the most abundant biomass on earth, and it consists of 70% sugar. However, it needs to be thermally, chemically, and biochemically processed before its microbial fermentation can happen (Peralta-Yahya, 2012). But second-generation biofuels also have some limitations. Second-generation biofuels are more difficult to produce and scale up (Albers, 2016). Chemical and enzymatic treatments are required for the transformation of non-degradable organic compounds into fermented sugars, which is costly (Kim, 2020).

Third Generation Biofuels

These third-generation biofuels are those that use algal biomass as their feedstock and are a good alternative to first- and second-generation biofuel feedstocks because they do not cause any food security conflict and have better fermentation efficiency (Abdullah, 2019). They can even be sophisticated on saltwater, and don't have land requirements like those of first- and second-generation biofuel feedstocks (Peralta-Yahya, 2012; Kim, 2020). Algae and microalgae are not composed of lignin. It is easy to decompose them for biofuel production (Kim, 2020). Algal biofuels are considered to play a vital role in accomplishing long-term climate policy objectives in the transport sector (Villareal, 2020). The limitations related to third-generation biofuels are that they have a very high initial cost of cultivation, require large carbon and nitrogen sources, and need solar energy, which is available only during the daytime. They do not have favorable economics, and biomass production is insufficient for commercialization. Therefore, fourth-generation biofuels were introduced to counter some of the limitations of third-generation biofuels (Abdullah, 2019).

Fourth Generation Biofuels

The fourth generation of biofuels is those that use genetically modified algae to accomplish improved biofuel production (Abdullab, 2019). The root of fourth-generation biofuels is the introduction of modified photosynthetic microorganisms using metabolic and genetic engineering approaches. Fourth-generation biofuels are still at a nascent stage in their development but are considered to be the best type of biofuel amongst all their alternatives. Third and fourth-generation biofuels are linked by the fact that genetic and metabolic engineering can be used for the same feedstock as third-generation biofuels (Figure 1) (Moravvej, 2019).

The fourth-generation biofuel has advantages like having a faster growth rate, efficient photosynthetic carbon dioxide fixation, reducing photo-inhibition (Abdullab, 2019). There is no need for any special culture media, and they can adapt to any place for cultivation, including sewage and saltwater (Moravvej, 2019). One of the main prospective properties of GM algae is increased light penetration. This can be accomplished by using a truncated chlorophyll antenna on the chloroplast. The limitations and risks related to GM algae-derived biofuels also require better attention. GMO algae have stringent regulations associated with them since they pose environmental and health-related risks. The major risk associated with the GM algae-derived biofuel is the residual water that remains after the harvesting process, which may contain plasmid or chromosomal DNA, which has the potential to cause lateral gene transfer.

Third and fourth-generation biofuels are not produced commercially yet because of their high production costs, inadequate biomass production, and environmental and health concerns (Abdullah, 2019).

Research and developments in Biofuel production: Technical aspects

The production of biofuels depends on biomass availability and, as the biomass resource and the land utilized for the production of biomass are limited, the efficient and sustainable conversion of biomass remains the most important issue (Müller-Langer *et al.*, 2014; Curtright *et al.*, 2012; Marten, 1982). One of the important criteria for the biofuel production chain is the efficiency with which biomass is converted into an energy carrier. The ratio between total output energy and total input energy describes the overall energy efficiency of biofuel production plants.

Energy or biofuel-driven biorefineries mainly involve the production of transportation fuels, power, and heat from biomass. The process residues may be used to produce biobased products, thus providing further economic and environmental benefits. IEA bioenergy developed a classification scheme for biorefineries consisting of four features, including platforms, products, feedstocks, and processes. Different biorefinery configurations can be described based on these features (Jungmeier *et al.*, 2013). The biorefinery concept adopts a wide range of technologies to produce biofuels and value-added products. Global energy demand is increasing daily, posing a significant risk to the environment (Cherubini, 2010).

Biological pathways and simulation for Biofuel Production

Utilizing various metabolic processes for isolating sub-metabolites for the production of biofuels, by exploiting existing pathways inside micro-organisms like bacteria, algae, fungi, and plant biomass, the processes are funneled and then subjected to downstream processing. Eco-friendly resources like plant and algal biomass (plant biomass-jatropha, rapeseeds, canola; algal biomass–chlorella sp.) and various microbial consortia have been researched extensively for the production of biofuels during the past decade (Dragone *et al.*, 2010; Heimann, 2016).

Plants and algae are rich sources of biomass that use their photosynthetic ability to store solar energy in the form of oil within seeds (in the case of plants) or large algal farms, which can be scaled up by pool cultivation (Voloshin *et al.*, 2015).

Biomass Potential

About 8.6% of greenhouse gas emissions come from the agriculture sector, released either during transportation of cultivated crops in the form of fossil fuels or as farming land waste like pesticides and chemical fertilizers. Agricultural residues have an estimated energy production potential of 9 billion barrels worldwide and 1 billion barrels in the USA alone.

Nonetheless, its economic utilization by efficient management and processing is a must (Lal *et al.*, 2004). The annual global primary production of biomass is equivalent to about 4,500 EJ of solar energy stored in a year (Sims, 2004). About 5% of this energy, or 225 EJ, would have covered almost 50% of the world’s total primary energy demand in 2006.

This 225 EJ corresponds to the primary annual sustainable bioenergy production of 270 EJ based upon economic production viability and the availability of free land for manufacturing (Hall & Rosillo-Calle, 1998).

From first- to modern-generation Biofuel production: Promising approaches

Conventional Industrial approaches

Thermochemical processes (direct combustion, gasification, liquefaction, and pyrolysis)

Biochemical processes (transesterification, fermentation, and biomethanation)

Enzyme-mediated processing

Production of first-generation biofuels

Globally, at present, the first-generation biofuels that are being produced and commercially utilized can be categorized into three major types, which include biodiesel, ethanol, and biogas. Biodiesel is produced through the transesterification of vegetable oils, residual oils, and fats. The chemical reaction of vegetable oils and animal fats with alcohol in the presence of homogeneous and heterogeneous catalysts gives rise to a mixture of methyl esters and glycerol. This product Fatty acid methyl esters (FAME) is known as biodiesel. For oils containing high amounts of free fatty acids (FFA), the basic transesterification process involving liquid acid or liquid base catalyst is not useful. For such oils, the use of solid catalysts is recommended, as these can simultaneously catalyze transesterification of triglycerides and esterification of free fatty acids to the methyl esters (Naik *et al.*, 2010; Kulkarni *et al.*, 2006; Meher *et al.*, 2006). Ethanol is produced from sugar or starch through fermentation. A variety of raw materials in the form of sugar, starch, and cellulose-containing crops are used for the production of ethanol through the fermentation process. Fermentation is defined as a metabolic process due to the activities of enzymes secreted by microorganisms involving chemical changes in organic substrates. Biogas is produced from the anaerobic digestion of liquid manure and other digestible feedstock. At the time of the anaerobic digestion of the biomass, in the absence of oxygen, anaerobic bacteria break down the organic matter and produce a mixture of methane and carbon dioxide gases, typically in the ratio of 70% methane to 30% carbon dioxide (Naik *et al.*, 2010).

Production of second-generation biofuels

For the production of second-generation biofuels, biomass used for the production of biofuels is categorized as homogeneous, such as wood chips; quasi-homogeneous, such as agricultural residues; and heterogeneous, such as municipal wastes. The biomass is made up of a diverse range of feedstocks, the most common of which are lignocellulosic biomass and others (Lee *et al.*, 2013). For converting biomass into second-generation biofuels, there are mainly two processes or technologies available: Thermochemical processes such as direct combustion, gasification, liquefaction, and pyrolysis.

Biochemical processes such as fermentation, and biomethanation

Thermochemical processes

For thermochemical conversion of biomass for biofuel production, feedstocks with a moisture content of 30% and a carbon to nitrogen ratio of greater than 30% are preferred (Pattanaik *et al.*, 2019). Heating of biomass under oxygen-deficient conditions direct combustion of biomass can be carried out in a well-ventilated area to provide an energy substitute for conventional fossil fuels when used in domestic stoves and boilers, with improved thermal efficiency and reduced emissions, with the only products being carbon dioxide and water (Kishore *et al.*, 2003; Naik *et al.*, 2010).

Biomass gasification involves the production of either producer gas or synthesis gas or syngas, which is a mixture of gases including carbon monoxide, hydrogen, nitrogen, and carbon dioxide in different proportions, by reacting biomass with air, oxygen, or steam. This involves a combination of thermochemical reactions, including reduction reaction, shift reaction, methanation reaction, and water-gas reaction. The process could be carried out through two routes: catalytic or non-catalytic (Kishore *et al.*, 2003; Naik *et al.*, 2010; Rowlands *et al.*, 2008; Lee *et al.*, 2014; Escobar *et al.*, 2009).

Liquidification is used to produce water-insoluble oils of high viscosity from biomass and requires the presence of solvents, reducing gases, and/or catalysts. The process can be carried out directly, i.e., direct liquefaction, or in the presence of solutions of glycerin, alkalis, propanol, etc. Pyrolysis of biomass involves the production of bio-oil, bio-char, and fuel gaseous products through the thermal decomposition of biomass by heat in the absence of oxygen. The pyrolysis process is subdivided into three classes depending on the different operating conditions, such as fast pyrolysis, conventional pyrolysis, and flash pyrolysis. Conventional pyrolysis is performed on massive pieces of wood. The process occurs with a slow heating rate, i.e., 0.1–1K/s, and with a residence time of 45–550 s. Fast pyrolysis occurs with fine particles of less than 1 mm in size. The process occurs with a fast-heating rate, i.e., 12–201K/s, a time range of 0.4–10s, and a temperature range of 845–1250K. (Shafizadeh, 1980; Naik *et al.*, 2010).

Biochemical processes

Liquid and gaseous products are produced from the use of biochemical techniques or microbes from organic products. Feedstocks with lignocellulosic compositions containing high moisture, i.e., > 30%, and a carbon to nitrogen ratio of 30 are preferred for biochemical conversion (Kishore *et al.*, 2003; Pattanaik *et al.*, 2019). This involves the breaking down of biomass into intermediates such as sugars, amino acids, or short-chain fatty acids by the activity of different microorganisms or enzymes, for conversion into liquid or gaseous fuels. In the context of the Indian scenario, two biochemical processes are of utmost importance: fermentation and anaerobic digestion. Fermentation is the process of conversion of simple sugars by yeasts into ethanol and carbon dioxide, while anaerobic digestion or bio methanation involves complex biochemical reactions, mainly acidification and methanation reactions, for producing biogas (Kishore *et al.*, 2003).

Production of third-generation biofuels

Different types of biofuels can be produced from algal biomass, which includes bioethanol, biodiesel, biogas, photobiological produced biohydrogen, etc. For deriving useful energy from algal biomass, the following processes or technologies may be utilized: biochemical, chemical, and thermochemical processes (Behera *et al.*, 2015). Production of biofuels from algal biomass relies on the lipid content of the microorganisms (Lee *et al.*, 2013). Up to 30 times more oil per acre can be produced from algal biomass as compared to other oil-producing crops. The higher oil yield from algal biomass makes it an interesting sustainable feedstock for biodiesel production. For the extraction of algal oil from algal biomass, different methods are available, which include mechanical extraction, enzymatic extraction, ultrasonic extraction, supercritical extraction, and chemical extraction methods. The algal oil is further processed via transesterification, usually, base-catalyzed transesterification, to convert it into biodiesel (Behera *et al.*, 2015; Bajhaiya *et al.*, 2010; Yun *et al.*, 1997).

Modern approaches: Biotechnological and Omics venture

Molecular marker technology

Second-generation biofuels are given preference over first-generation biofuels because using the first-generation feedstock for biofuel production could lead to food shortages. But the second-generation biofuels also pose a problem, and that is the need for the fertile land. Rather than using conventional approaches, molecular marker approaches can prove to have immense potential. Several plant species have been identified as sources of biofuels (both biodiesel and bioethanol). *Jatropha curcas* L. has been identified as a major source of biodiesel mainly due to its highly adaptive features, which are supposed to be advantageous in resource-limiting environments. Also, for the genetic improvement of any species, information about its genetic background is essential. Genetic variations in *Jatropha curcas* in India have been studied by using Amplified Fragment Length polymorphism (AFLP), Randomly Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR), and Directed Amplification of Minisatellite-region DNA (DAMD) techniques have shown detailed RAPD and AFLP analysis of *Jatropha curcas*. So, the germplasm characterized by the above studies could be employed in the breeding programs, leading to the genetic improvement of the species through MAS and QTL analysis, which would finally lead to the improvement of the bioenergy crop (Yau, 2018). The *Miscanthus x giganteus* genetic variations have been studied by using several marker systems such as RFLP, RAPD, inter-sample repeat-polymerase chain reaction (ISSR-PCR), and sequence-related amplified polymorphism (SRAP). These studies facilitated conservation, association analysis, and biological discovery for improved Miscanthus as a bioenergy crop (Moore *et al.*, 2014). For example, in maize, using crops for extracting biofuels such as bioethanol is becoming a viable alternative to fossil fuels. As one of the major crops, maize offers promise in this regard. Advantageous in comparison to other crops, maize can provide both starch (seed) and cellulosic (stover) material for bioethanol production. The usage of one crop for food, feed, and fuel has raised concern over land segregation and crop distribution based upon its mass versus energy generation value.

Ensuring the optimum process involving an eco-friendly infrastructure layout would ensure efficient ethanol production. Conventional breeding, molecular marker-assisted breeding, and genetic engineering are already acting as tools in biofuel production (Figure 1) (Ahmed *et al.*, 2021).

Novel approaches like synthetic metabolic engineering and subsequent techniques for predicting novel metabolites like machine learning algorithms offer a wide range of possibilities for enhanced bioethanol production from maize.

Targeted mutagenesis

Targeted mutagenesis is an essential tool of reverse genetics that can be used experimentally to investigate basic plant biology or modify crop plants for the improvement of important agricultural traits (Alan Lloyd, 2005). In regards to the prospects of biofuels, there are many reports which revolve around targeted mutagenesis for improvised production of a particular substance of importance (or its precursor) or decreased production of a toxic substance (Ruffing, 2013). Described one example of how this technique can be helpful in biofuel production. Free fatty acids (FFAs) are an important biofuel precursor, but the free fatty acid produced inside cyanobacterium is also responsible for the generation of reactive oxygen species inside the cyanobacteria, which leads to increased permeability of the cell membrane. So, there is a need for free fatty acids for biofuel production, but there is also a need to overcome the negative effects generated due to the free fatty acid production inside the cyanobacteria. To solve this problem, the genes responsible for this toxic effect were found using RNA-seq analysis of the cyanobacterium. The gene knockout of two prions and the overexpression of ROS-degrading proteins and hypothetical proteins lead to reduced toxic effects of FFA production as well as a minor increase in FFA yield. 15 genes were targeted for reduction of toxic effects, and single gene-targeted mutagenesis led to a minor increase in FFA production (Ruffling, 2013). Similarly, in cellulosic biofuels, acetic acid is generated during pretreatment and hydrolysis of the feedstock. Acetic acid could significantly inhibit the fermentation efficiency of the microbe. Hence, microbes should be engineered in such a way that they can tolerate a high concentration of acetic acid. Using a multiplex mutagenesis strategy, the researchers were able to achieve the desired results (Wu, 2019). In the above two examples, it was seen that the same approach to mutagenesis was used in two different manners. Similarly, the approach of mutagenesis can be utilized in many different ways (Bessadok, 2019). Reported that ethyl methanesulfonate (EMS) mutagenesis drives the cell viability of *Sarcomeres cerevisiae* to 43% if exposed to EMS for 45 minutes (Connor, 2010). Demonstrated the mutagenesis approach for developing an improved strain of *Escherichia coli* that could produce 3-methyl-1-butanol by selective pressure for L-Leucine biosynthesis. So, amongst all the advanced approaches, the targeted mutagenesis approach holds intangible value in increasing the efficiency of biofuel production.

Synthetic Biology

Synthetic biology uses engineering principles for biological analysis, to modify existing systems or create new ones (Wang, 2019). The root of synthetic biology lies in creating artificial regulatory circuits that can direct cellular behavior based on user-defined inputs to produce desired outputs like biofuel production, chemical production, protein production, etc (Jagadevan, 2018). The fourth generation of biofuels mainly depends on this technology (Scaife *et al.,* 2015; Berla *et al.,* 2013; Hays and Ducat, 2015). Systems biology, though in an earlier research phase, has proved the possibility of integrating synthetic genetic expressions into plants (De Lange *et al.,* 2018). Using synthetic abscisic acid receptor, a strain of A. Thaliana has been engineered can survive in water scarcity conditions due to chemical induction (Park *et al.,* 2015).

Transgenic Arabidopsis with (OYE3) gene overexpression bleaches in the presence of trinitrotoluene (TNT) in soil. This can be used as an effective method of phytoremediation and subsequent energy generation. The genetic engineering approaches for industrial production of biofuels and other associated secondary products will imply a combinatorial approach of metabolic engineering and synthetic biology shortly, more appropriately termed "synthetic metabolic engineering (Pouvreau *et al.,* 2018). Henceforth, our focus in this review is to discuss a few strategies adopted to improve photosynthetic organisms as the cost-effective roadmap toward establishing biofuel refineries employing advanced transgenic technologies to improve their productivities

Metabolomics-assisted synthetic Biology

The goal of generating the desired material with a decent conversion rate from the substrate to product, which is one of the major goals of synthetic biology, has its roots in metabolic engineering. In metabolic engineering, to increase the production of a particular substance inside the cell, the pathways that are predicted to be related directly to the production of that substance are optimized inside the cell (Ellis, 2012 and Chang 2012). They engineered yeast with a five-cassette assemblage. A tool, Promoter-based Gene Assembly and Simultaneous Overexpression (PAGSO), which involves overlapping of oligonucleotides for recombination assembly with separate promoters, was developed for the same. Applying PAGSO, they made a new strain of yeast that could simultaneously express three distinct cellulase genes. Engineered Escherichia Coli using the tools of synthetic biology was successful. The engineered strain was able to produce cadaverine with rapid assimilation of galactose, which could be an effective future feedstock. The responsible pathway was hugely expressed to pass the flux to cadaverine production using synthetic biology tools (Kwak, 2017).

Next-generation sequencing technology

Since the discovery of the structure of DNA, a lot of advances have been made in understanding the complexity and diversity of the genomes of living organisms (Goodwin, 2016). Deciphering the DNA sequence is the most comprehensive way of learning about a living organism's genome (Berglund, 2011). The automated sequencing method is often referred to as "First Generation Sequencing." (Wirth, 2012). For years, Sanger sequencing has taken credit for being the gold standard in sequencing technology (Berglund, 2011). Next-generation sequencing (NGS), massively parallel or deep sequencing are related terms that describe a DNA sequencing technology which has revolutionized genomic research (Behjati, 2013). Next-generation DNA sequencing has the potential to dramatically accelerate biological and biomedical research by enabling the comprehensive analysis of genomes, transcriptomes, and interactomes to become inexpensive, routine, and widespread, rather than requiring significant production-scale efforts (Shendure, 2008). These NGS technologies help in the speedy determination of DNA sequences and also help in the assembly of massive databases of short sequence fragments and bioinformatics analysis of the results (Wirth, 2012). Some of the techniques that fall under next-generation sequencing technology are 454 pyrosequencing, HiSeq sequencing technology, and SOLiD. 454 Life Sciences, which is a Roche company, introduced the first-ever next-generation sequencing technology to the world. Its overall procedure was brought about in 2005. Illumina, the company that produces the Illumina sequencing platforms like GA/Hiseq/MiSeq, which are the most used family of platforms, was introduced in 2006. In 2007, Applied Biosystems launched SOLiD, which was not as popular as Illumina or 454 but has utility in different microbiome applications. A comparison of all three and the Sanger sequencing technology is presented in the Table 1. (Hodkinson, 2005).

Applications

With the fast NG-sequencing technologies, it is possible to determine the diversity of a microbial community within an environment (Wirth *et al.*, 2012). have worked on the characterization of the microbial community using solid technology. They deciphered that the major microbes responsible for organic matter decomposition include the members of the Eubacteria, and Archaea are present in a very small number. Not only this, but the determination of the diversity of different types within the overall diversity of Archaea and Eubacteria is also possible and that is what this research attained (Wirth *et al.*, 2012 and Zhu, 2013). The authors provided a broad view of the complex molecular mechanisms of Synechocystis sp. PCC 6803 against butanol stress and also acknowledged the genes that could be used to engineer the cyanobacterium against butanol stress. This study used next-generation RNA sequencing technology and was successful in conducting a comprehensive transcriptomic, metabolic, and proteomic analysis for the identification of gene targets. For the same organism, ethanol-resistant genes were identified by (Wang, 2012). Wang used next-generation sequencing technology and performed analysis through quantitative reverse-transcript PCR (RT-PCR). The cellular responses and hence the gene targets for ethanol tolerance were identified. Furthermore, next-generation sequencing was used to determine the transcriptome of Lemna aequinocitalis 6000 responded to nitrogen starvation on different days, with sucrose as the exogenously applied source. It was seen that the genes involved in nitrogen metabolism showed the earliest response, and those involved in carbohydrate biosynthesis showed a delayed response to nitrogen starvation. It was concluded that ADP-glucose and starch content increased as a result of gluconeogenesis and the TCA pathway, and lipid and pectin biosynthesis were deceased (Liu, 2012).

Metabolic Engineering

Metabolic Engineering is a set of techniques that involve the modification of a cell's biochemical reactions by introducing new ones to improve cellular properties or cellular product formation (Bailey, 1991). Engineering is the major tool and research area under the focus of scientists and researchers used metabolic engineering to increase the yield of any product produced metabolically by microorganisms (Sheng *et al.,* 2017). Metabolic engineering requires the identification of enzymes and metabolic pathways that require improvement or replacement. This process of identification requires advanced computational efforts (Long *et al.,* 2015). Proteomic studies are therefore used for designing metabolic models at the genome level and for the optimization of novel metabolic pathways in microbes used the technology to develop kinetic models of *P. putida*. These kinetic models helped them predict metabolites produced in a particular pathway and also engineer the changes in the pathways for improvised production of the biochemical. With predicated reactions in the model being 775 and predicted metabolites being 245, they applied a new set of constraints to the model. These constraints were within the thermodynamic-based flux analysis that led to the compartmentalization of the metabolites. Through gap-filling and thermodynamic curation, they were able to reduce the model, and they finally created three models that described the central carbon metabolism of the organism. In these models, Tokik and coworkers observed the experimental responses of many different gene knockouts, and also developed models with engineered metabolic changes that would make the organism more tolerant of the increased ATP demand (Yao *et al.,* 2014). Introduced a fatty alcohol-producing pathway into photosynthetic cyanobacteria Synechocystis sp. This was achieved by expressing the fatty acyl-Coenzyme A (acyl-CoA) reductase gene heterologously. One problem that occurred was the accumulation of hydrocarbons, which was tackled by the knockout of the aldehyde-reformulating oxygenase gene and redirecting the carbon flux due to this accumulation to the newly introduced fatty alcohol-producing pathway. The productivity of fatty alcohol was increased in this way. Fatty alcohols are considered important industrial chemicals, biofuel additives, as well as potential biofuels (Yao, 2014 and Liu, 2014). Similarly, they were the first to use metabolic engineering to delete the L-lactate dehydrogenase gene in *C. bescii*. Similarly, there are various examples and ways in which metabolic engineering is used to attain an increased production of a particular metabolite in the network, or for flux transfer for efficient use of the flux (Cha, 2013).

Functional Metagenomics Technology

Metagenomics technology comprises DNA isolation straight from the target environmental sample (Bastien, 2013). Metagenomics-based approaches have bypassed cultivation-dependent approaches, allowing us to directly study the diversity of genes and analyze the biochemical characteristics of diverse microbial communities. It has the potential to determine the sequences of enzymes whose sequences are unknown to date (Li, 2011). Functional metagenomics is an approach that sanctions high-resolution genomic analysis of microorganisms present in an environment while linking them to specific functions in that environment (Chistoserdovai, 2010) In functional metagenomics, there is a need for screening procedures that locate the function and/or enzymes of interest in the environmental sample (Bastien, 2013). It has led to the discovery of various industrially important enzymes from both natural and artificially engineered ecosystems (Yang, 2016).

It is better than the conventional shotgun method because there is a radical reduction in the amount of sequence analysis involved and a considerable increase in the amount of information related to the targeted family of functions (Bastien, 2013).

With the integration of omics technology with bioinformatics tools, functional metagenomics can recognize discrete traits employed by microbes that vary across time and space for their survival (Johnson, 2017). Enzymes like cellulase have wide-ranging applications in the biofuel industry. Cellulase efficiently converts cellulosic substrates into fermented sugars (Srivastava, 2018 and Yang, 2016). They discovered novel cellulose enzyme-producing genes cel7482, cel3623, and cel36 in anaerobic beer.

Similarly, hemicelluloses have a vibrant role in biomass hydrolysis. They used this technology for mining hemicellulases in the gut microbes associated with the fungus-growing termite Pseudacanthotermes militaries and were able to find the ability of the same microbiome to degrade biomass components like arabinoxylans and arabinans (Wang 2016).

Worked with the same technology in microbial consortia enriched with compost and discovered a new standard for the microbial deconstruction of lignocelluloses by underlining the role of thermophilic actinobacteria in lignocellulose biodegradation processes.

Genome editing technology

Engineered Nucleases

Genetic engineering involves the successful conveyance of genetic material to the genome of an organism, which results in genetic modifications (Figure 1). Successful delivery of genetic material to animal cells via transfection by viral DNA was achieved in the early 1970s. For plants, the successful transformation was achieved a decade later, and for algae, the transformation was achieved in the late 1980s (Jeon, 2017). Many strategies are utilized for genome editing, some of which are Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) – CRISPR Associated Protein 9 (Cas9), Transcription Activator like Effector Nucleases (TALENs), Zinc Finger Nucleases (ZFNs), and Homing Endonucleases (Gaj, 2016).

CRISPR/Cas9 system

CRISPR Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats)– Cas system exists in prokaryotes as an RNA-mediated immune system that protects the prokaryote from bacteriophages (McGinn and Marraffini, 2016). Genome editing in organisms like bacteria, yeast, and even mammals can be done by the use of the Type II CRISPR-Cas9 system (Jako_ci_unas, *et al.*, 2015). The CRISPR-Cas9 system can be successfully utilized for the improvised production of metabolites, chemicals, and biofuels (Citorik *et al.*, 2014). The production of cheese, alcohol, and bread is done by a common process known as fermentation. Yeast is the common agent used inside a bioreactor (on an industrial scale) or vessel (domestic). During the biochemical processes of biofuel production that occur inside the fermenter, yeast undergoes stress mechanisms that generate excessive protein or metabolites for the breakdown of complex substrates. These metabolites cause harm to the yeast cell. Recent advancements in genetic engineering have paved the way for scientists to reduce the toxicity effect of these metabolites by using the CRISPR-Cas9 gene alteration and cleavage method. The research aims at single gene alteration in yeast to make it resistant (i.e., tolerant) against these harmful metabolites. Genetically modified bacteria also do biofuel generation.

Acetogenic microbes such as Clostridium autoethanogenum act as fermenting agents for the fermentation of biogas. Before the discovery of CRISPR as a gene-editing tool, the genetic and molecular level study of biochemical processes involved in the production of bioethanol by acetogenic microbes was challenging. Limited knowledge about the mechanisms makes it a great task for researchers to know about the yield capacity of the bacteria.

With the advent of CRISPR, knock-out studies of genes in these bacteria could be efficiently done. CRISPR-Cas9 technology is the most commonly used technology for genome editing nowadays. Table 2 provides a list of different organisms, including bacteria, fungi, plants, and yeast, in which genetic modifications have been performed using CRISPR-Cas9 technology.

There are many more developments in genetic engineering that revolve around the technology of CRISPR are homology-directed repair is mediated by the CRISPR–Cas ribonucleoprotein (Naduthodi, 2019; Yoshimitsu, 2018).

Homing Endonucleases

Homing is the lateral transmission of an intervening sequence to a homologous allele that lacks the sequence. Homing endonucleases are small proteins of less than 40 kDa, which is a decent advantage (Chevalier and Stoddard, 2001). These enzymes make extremely sequence-specific contacts with the DNA. And thus, they typically show extreme specificity. But the lack of readily accessible, customizable reagents and unclear guidelines for making a construct that identifies a specific DNA sequence rules out its existence as an attractive choice for genome editing in the long run (David *et al.*, 2014).

Zinc Finger Nucleases

Increasing demand for photosynthetically derived biofuel precursors has led to the exploration and genetic modification of metabolic pathways in plants and algae. Zinc finger nucleases have two chimeras, and each chimera can recognize a specific DNA sequence, and these sequences are close to each other in location. These chimeras bind to DNA and cut the DNA such that the fragment present in between the two regions of binding is released.

This naturally causes the cellular repair machinery NHEJ to activate. So, here, the NHEJ can result in indels of various sizes, which could result in a non-functional protein on translation, or it can use a fragment of DNA supplied to the cell externally and finally, this would lead to the replacement of the sequence between the two cut sites by homologous recombination (Costa, 2017). However, the inability of ZNFs to edit target-specific genomes is their main disadvantage (Kim, 2010).

TALEN

In recent studies in rice, bacterial blight resistance disease was obtained using TALENs, mutations of the Os11N3 gene responsible for bacterial blight resistance susceptibility (Li *et al.*, 2012). TALENs are derived from Xanthomonas. They have a central repeat domain that facilitates DNA recognition.

TALENs can act as site-specific endonucleases for selective genomic cleavage. As such, it is extremely specific and, unlike ZNFs, each repeat domain in TALENs recognizes a single base. TALENs would have been the leading technology for genome editing, but it was short-lived as CRISPR-Cas9 was discovered shortly thereafter (Miller, 2011).

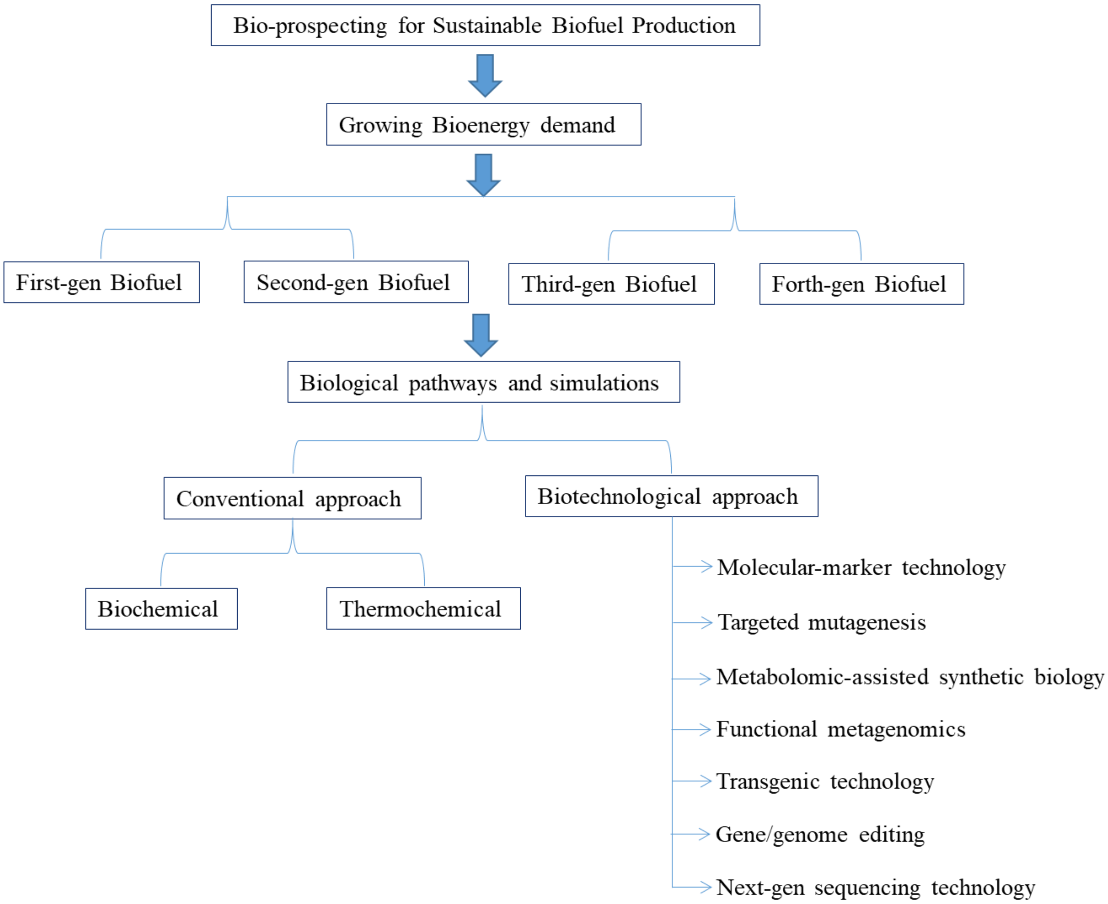
Table.1 A comparative analysis of different sequencing platforms which can be used for bio-prospecting of biofuels

S. No.	Particulars	454 GS FLX	HiSeq 2000	SOLiDv4	Sanger 3730xl
1.	Sequencing mechanism	Pyrosequencing	Sequencing by synthesis	Ligation and two-base coding	Dideoxy chain termination
2.	Read length	700 bp	50SE, 50PE, 101PE	50 + 35 bp or 50 + 50 bp	400~900 bp
3.	Accuracy	99.9%*	98%,(100PE)	99.94% *raw data	99.999%
4.	Reads	1 M	3 G	1200~1400 M	—
5.	Output data/run	0.7 Gb	600 Gb	120 Gb	1.9~84 Kb
6.	Time/run	24	Hours 3~10 Days	7 Days for SE 14 Days for PE	20 Mins~3 Hours
7.	Instrument price	Instrument $500,000, $7000 per run	Instrument $690,000, $6000/(30x) human genome	Instrument $495,000, $15,000/100 Gb	Instrument $95,000, about $4 per 800 bp reaction
8.	Cost/million bases	$10	$0.07	$0.13	$2400

Table.2 Details of multi-omics platforms used for bio-prospecting of biofuels

S. No.	Organism	Achievement	Reference
1.	*Ogataea polymorpha*	Biosynthesis of Resveratrol improved (20 folds), and biosynthesis of human serum albumin and cadaverinewas achieved.	Wang, 2018
2.	*Clostridium autoethanogenum*	Gene expression fine-tuning: Improvement in desired gene deletion achieved (50 %).	Nagaraju, 2016
3.	*Bacillus subtilis*	Improved efficiency for gene deletion, gene insertion, site-directed mutagenesis, large DA fragment deletion, and 3 simultaneous point mutations.	Liu Dingyu, 2019
4.	*Myceliophthora thermophila*	Hyper cellulose production was achieved, lignocellulose activity & extracellular protein secreted increased. multigene disruption achieved.	Liu Qian, 2017
5.	*Ralstonia eutropha*	Increased electroporation efficiency (First genome editing method based on electroporation-based CRISPR-cas9 method in *R. eutropha*)	Xiong, 2018
6.	*Brassica Napus*	An improvedgene-editing approach was developed. Decrease in oil content, and increase in the size of oil bodies.	Zhang, 2019
7.	*Kluyveromyces marxianus*	Disruption of ethyl acetate biosynthesis genes and complete elimination of ethanol production, and so the accumulation of acetaldehyde.	Löbs, 2017
8.	*Panicum virgatum*	Establishment of CRISPR Cas9 system, reduced cell wall thickness, reduced lignin content, increase in glucose and Xylose.	Park J, 2017

Fig.1 Tale of bio-prospecting for sustainable biofuel production from conventional to modern biotechnological approach



Other modern biotechnological multi-omics platforms

Transplastomic technology

Chloroplasts, which are the photosynthetic organelles of plant cells, hold a small circular genome called a plastome. The plastome is highly polyploidy, and the transcriptional and translational machinery of the chloroplast is similar to that of prokaryotes. This gives plastome transformation an edge over nuclear genome transformation (Kolotilin, 2013). Chloroplast transformation has the advantages of site-specific gene integration, operon implanted multiple gene expression, transgene containment, and it allows a high level of foreign protein accumulation. Furthermore, the chloroplast stands as an ideal candidate for the expression of numerous cellulolytic enzymes from mesophilic and thermophilic fungi and bacteria (Castigila, 2016).

When compared to the cytoplasm, chloroplast can accommodate a greater number of foreign proteins (Jang *et al.,* 1999; Nawrath *et al.,* 1994).

An example of the improvement of foreign protein accumulation in the chloroplast has been presented by (Richter *et al.,* 2018). They were able to successfully enhance the foreign protein accumulation of cellulase (Cel6A) in the transplastomic *Chlamydomonas reinharditi* (Castigila, 2016) achieved a very high production level of thermophilic and hyperthermophilic enzymes. They concluded that plastid transformation is a very economical cellulolytic enzyme production platform.

Promoter Engineering

High levels of expression are not always desirable from a gene, thus control over its expression level is important. This control can be achieved by having different promoters of different strengths available for the gene Promoter engineering is considered the most effective and resourceful way of fine-tuning the control of transcription in Escherichia Coli, *Bacillus Subtilis*, and *Cornybacterium glutamiccum*. Used the promoter engineering approach on the xylose utilization pathway and optimized the expression levels of Xylose reductase, Xylitol dehydrogenase, and Xylulose kinase (Hammer, 2006; Nevogit, 2006; Shiue, 2012; Blazeck, 2013; Guiziou, 2016; Gilman, 2016; Watanabe, 2017; Gao, 2016).

Gene Silencing

Gene silencing is referred to as the epigenetic modification of gene expression, which leads to the inactivation of genes or larger chromosomal segments that were previously active. For transcriptional inactivation, the mechanism can be DNA methylation or histone modification, which would lead to chromatin compaction, so that it becomes inaccessible to the transcriptional machinery. Apart from these, small interfering RNAs (siRNAs) can also be used for transcriptional gene silencing. For posttranscriptional gene silencing, mRNA degradation, repression of its translation, or both the mechanisms together can be used. The posttranscriptional modification can be mediated by small interfering RNAs (siRNAs), microRNAs (miRNAs), and double-stranded RNAs (dsRNAs) using the gene-silencing technology on Jatropha curcas (Filipowicz, 2013 and Kim, 2014). The oil content of *Jatropha curcas* is one of its important traits as a biodiesel crop. It uses the RNAi technology for silencing a gene, JcSDP1, in Jatropha. This gave rise to a 13–30% increase in storage lipid accumulation in the seeds. Furthermore, the free fatty acid (FFA) content in seeds was reduced from 27% in control to 8.5% in RNAi-manipulated plants.

Biosafety issues: Environmental and socioeconomic impact of Biofuels

The demand for biofuels is increasing, and the reasons behind it are the increasing oil prices, climate change, and energy security apprehensions.

There are also concerns regarding the social and economic implications of utilizing biofuels as a major fuel source. On the one hand, biofuels can be a great incentive to rural economies, but on the other hand, aspects like a threat to food security and uncertainty regarding land availability in populous countries are also noteworthy. Even though biofuel production is minor in comparison to energy demands, it is significant in terms of agricultural production. Agriculture has some undesirable effects on biodiversity, but biofuels, these negative effects would be of particular concern (The state of food and agriculture, 2008).

One of the major concerns that fossil fuels pose is the emission of greenhouse gases, and as far as biofuels are concerned, the production of biofuels has a very small amount of greenhouse gas emissions. The bioenergy crops also lessen the carbon dioxide present in the environment by taking it up and passing it on to the crop biomass or soil. Since biofuels promise to provide multiple benefits to the social, ecological, geographical, and economic domains, their popularity and production have increased and they have attracted more investment than ever. Countries and their policymakers are focusing on encouraging this fast-catching industry (Timilsina and Shrestha, 2010). There are many social and ecological benefits of biofuels that have been declared by big industries (Goldemberg *et al.*, 2008; Goldemberg and Guardabassi, 2009).

And many organizations have questioned these benefits. There are positives and negatives regarding the impacts of biofuels. It's the policymakers who have to decide what could prove to be the best socially, economically, and ecologically for their country and region (Civil Society Biofuels Forum, 2010).

Costs, prices, and economic impacts of biofuels

The major cost involved in the production of biodiesel is the feedstock. The cost of feedstock production is about 75–80% of the total operational cost, and labor costs, the cost of methanol and catalysts add to it (Haas *et al.,* 2006). To decrease the cost of biodiesel production, we need to focus on increasing the yield of bioenergy crops and find ways to make use of the by-products of the production process, like glycerol. Other ways to do so can be the use of co-solvents like tetrahydrofuran, which is known to reduce the processing costs (Demirbas *et al.,* 2008). All this can be done to reduce the cost of biodiesel production, but still, it is not enough to have a significant impact on the overall costs. The feedstock chosen for its production, the process used for conversion, the scale of production, and the region where it is being produced all play an important role. If we focus on ethanol, it is costlier than gasoline in most parts of the world where it is manufactured. Taking into account the region of Brazil, we can see the price of ethanol giving a bit of competition to the price of gasoline. Even the ethanol produced in Brazil using sugarcane is cheaper than the ethanol being produced in the US using corn, which is cheaper than the ethanol being produced in Europe using gran and sugar beet. So the cost of the final product depends on a lot of factors. It is interesting to note that the cost of large-scale production of biofuels is much higher than the cost of petroleum products in developed countries, whereas in developing countries the cost of biofuel production is lower than the price of petroleum products (United Nation, 2006).

Environmental impacts of biofuels

By 2040, there will be 28 percent more demand for energy as compared to today's demands (Osman, 2021). With the depletion of fossil fuels, researchers are looking for an alternative energy source, for which biofuels are also a great contender (Gaurav *et al.,* 2017). Although there is a widespread belief that the inclusion of biofuels in a country's energy chains would lead to fewer greenhouse gas emissions overall, policymakers should still assert the sustainable production of biofuels, and the policies regarding biofuels should be strictly based on evidence and must not be based on beliefs (Osman, 2021). Greenhouse gases are emitted from fossil-based fuels, which cause severe environmental problems like global warming. Biofuels have become an increasingly popular area of research because scientists want to develop a fuel that does not result in extensive emissions of greenhouse gases, as is the case with fossil-based fuels. However, the United Nations (UN) Food and Agricultural Organization (FAO) argues that biofuels have unintended negative impacts on land, water, and biodiversity. Agriculture, which some argue is the artificial method of obtaining food from plants, has already had a devastating impact on an ecosystem's biodiversity and environmental factors, to the point of extinction. If we shift our dependence on biofuels from fossil-based fuels, the result would be an increased load on the local environment, and the side effects would be disastrous. An argument supporting biofuels is that there is at least one greenhouse gas emission linked to their products and that the crops would act as a sink for the carbon dioxide already present in the environment. However, some scientific studies have shown that the feedstock grown for biofuel production has specific adverse environmental effects. It has been mentioned in the report mentioned above that, subject to the production method chosen, certain crops could generate more greenhouse gases than fossil fuel does. So, looking at the bigger picture, it can be argued that the production of biofuels is quite certainly not very good for the environment (The State of Food and Agriculture 2008 report).

Finally, it has been shown that first-generation biofuels are efficient in terms of energy and production; on the other hand, they are not suitable for reducing greenhouse gas emissions. There are fewer overall greenhouse gas emissions in third-generation biofuels, so they might look lucrative for the environment, but their production needs a lot of energy, which comes from fossil fuel-powered electricity. So, third and fourth-generation biofuels are the only ones that could be relied upon for the future of energy. More research on them should be done, leading to the discovery of novel ways for their production (Ayusmita Ray, 2021).

Prospects

Most traditional biofuels, such as ethanol from corn, wheat, or sugar beets, and biodiesel from oilseeds, are produced from classic agricultural food crops that require high-quality agricultural land for growth. Ethanol is a petrol additive or substitute. Production of ethanol from biomass is one way to reduce both the consumption of crude oil and environmental pollution.

By 2050, the global aviation industry plans to reduce its emissions by 50% through improvements in air traffic management, aircraft design and promoting the commercial usage of biomass-based fuels. At present, it can be stated that biogas production is more energy-favorable in comparison to the process that involves the combination of biogas and bioethanol production.

Sensitivity analysis is crucial to testing the robustness of the results obtained using LCA. LUC defines the release of sequestrated carbon into the atmosphere when carbon-rich lands, such as forest lands, are converted to low carbon-content lands, such as agricultural areas, for the growth of oilseed crops, and this release of carbon creates carbon debt and increases the annual energy usage in the biofuel industry. It is evident that the development of biofuels from biomass is still in rudimentary stages and considerable research work is essential for the optimization of biofuel production costs and energy utilization.

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