



Advances in Biofuel Production : Current Scenario and Challenges

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Abstract

Current review gives the snapshot of classes, revolution and challenges in biofuel production to replace the fossil fuels. Biological production of biofuels are capturing the world market due to limitations of petroleum based fuel. Researchers are now more attracted to explore new technologies for biofuel production. Biomass for biofuel production is one of the alternative; due to its renewability, low CO₂ and less GHG emission etc. The main problem regarding biomass use is efficiency to replace complete fossil fuels in near future. For biomass generation, land use causes ethical issue which results in increasing food prices. The biggest scientific insight to rely on new technologies like Synthetic Biology, Metabolic Engineering and Photovoltaic's in which engineering of biological systems are possible for the betterment of yield. These upcoming technologies will fulfil the requirement of energy, decrease the costs of fuel and will be able to solve environmental issues too. In photovoltaic technology solar system will be directly utilised by genetically engineered photosynthetic microorganisms or completely synthetic factories. Other than solar systems we may use geothermal energy to hit the bacterial photosynthetic machinery for direct biofuel production.

Keywords: Solar biofuels, Genetically engineered microorganisms, Biomass

Introduction

Biofuels are considered to be the most promising in the short term as their market maturity is above than other options (T. Wiesenthal et al 2009) . The European Council in March 2007 endorsed a mandatory target of 20 % share of energy from renewable sources in overall energy consumption by 2020. It is mandatory that 10 % of minimum target to be achieved by all states member for the share of biofuels in the transport sector by the year 2020 (EU, Directive 2009/28/EC).Traditionally vegetable oil is used to make diesel,was firstly found by E. Duffy and J. Patrick in 1853 (Feofilova, E. P et al 2010) .Currently used common feedstock's for biodiesel production are vegetable oils derived from edible oil crops, such as rapeseed, palm, soybean and sunflower. However, biodiesel from edible oils is arguable and has called Crime Against Humanity (Ferrette G. 2007). It is needless to mention that biodiesel seems currently economically competitive according to the latest Clean Cities Alternative Fuel Price Report (2015) released by the United States Department of Energy (Clean cities Alternative fuel prize report 2015) .

Global climate change linked to the accumulation of greenhouse gases causing concerns regarding the use of fossil fuels as the major energy source. To mitigate climate change, one solution is to rely on the ability of microorganisms to use renewable substrates for biofuels production (James C et al 2016). Humankind has seen tremendous progress in different industrial sectors like pharmaceutical, cosmetics, textiles, fertilisers, petrochemicals and synthetic chemicals. This enormous growth contributes to pollute the environment by out letting the harmful chemicals and highly toxic intermediates leading to water and air pollution. Microbial biotechnology gives best solutions with environmental friendly approach, by identifying the indicator microbial strains, causative agent for the problem and implementing the useful ones for environmental bioremediation. The microbial associated bioremediation process is benefited to the society by exploiting the metabolic capabilities of microorganisms. Microorganisms are capable to utilise the toxic compounds and transform in to utilizable intermediates and products. Agro-industrial waste products or municipal waste water conversion technology in to industrially useful value added products has already been exploited as an attractive alternative for biofuel production (J. Joshi Sanket 2016) .

Due to depletion of global petroleum and its increase in price, biodiesel is becoming one of the most promising global energy market in the recent years. Researchers are now exploiting the oleaginous microorganisms as an alternative source for biodiesel production. Currently, they are focusing on reducing the production costs by searching waste materials as substrates wherein entire wastes can be utilised for energy production (Dayana Montero-Rodríguez et al 2016) .Biomass is one of the best source for biofuel production; earlier it was used only for cooking and heating purpose. To minimize the complexity of biomass transportation and storage , to avoid harmful effects of direct combustion one needs to advice its conversion into biofuels (Baratieri, M 2008) . Biomass can be used to produce biofuels via different thermo chemical and biochemical processes such as biomethanation, fermentation, pyrolysis and gasification (Verma, M 2012 and Akia, M 2014) . One of the most efficient and promising way for biofuel production is transesetrification reaction. Conversion of

mixtures of triglycerides like vegetable oil and animal fats with methanol or ethanol in presence of acids, alkali or enzymes as a catalysts with the formation of fatty acid methyl esters (FAMES) .

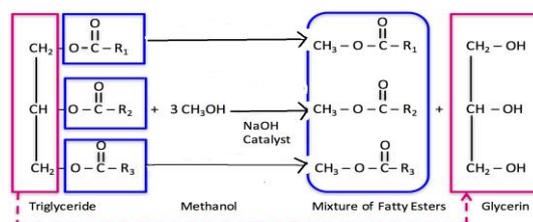


Figure 1. Transesterification reaction

There are various methods for biodiesel production from WCO (waste cooking oil) that can be divided into three main groups: (a) homogeneous, (b) heterogeneous (c) non-catalytic transesterification. Several types of catalysts have been widely used for biodiesel production. Base homogenised catalysts face major challenges in terms of free fatty acids and water content of the oil. To overcome this challenge two step process is widely implemented (Farooq M 2013 and Yaakob Z et al 2013) . Alkali-catalyzed transesterification method is generally preferred which includes the use of a homogenous catalyst such as sodium hydroxide or potassium hydroxide etc. Different levels of catalysts and alcohols have been employed to obtain the highest yield from different types of oils (Y.C. Sharma 2008). The transesterification reaction is controlled by three mechanisms: mass transfer, kinetic and equilibrium. The mass transfer becomes slow or poor if the two reactants are immiscible (i.e. methanol and triglycerides). On the completion of the mass transfer, the resulting process is controlled by the kinetic. Both kinetic and mass transfer of the reaction get ameliorated by increasing the reaction temperature and by vigorous mixing (G. Vicente et al 2005). Vigorous mixing results in to increase in the rate of collisions between the reactants thereby homogenization of the reaction mixture occurs (Z. Helwani 2009) .

One of the most promising renewable biofuel is biodiesel. Biodiesel is biodegradable, nontoxic and has similar properties to conventional diesel which becomes the choice for fuel consumption (Liang M.H. and Jiang J.G 2013) . In terms of energy security, prevention of climate change and rural development; Biorefineries gives priority to renewable substrates for bioenergy, biofuel and biochemical production over to chemical refining of petrochemical feedstocks (Cherubini F. 2010) .

Classes and Types of Biofuels

Biofuels are broadly classified as primary and secondary biofuels. The primary biofuels are used an unprocessed form for heating, cooking or electricity production, such as fuel wood, wood chips and pellets, etc. While secondary biofuels are produced by processing of biomass, e.g., ethanol, biodiesel, DME, etc., that can be used in vehicles and various industrial processes. The secondary biofuels are further divided into first, second and third generation biofuels on the basis of raw material and technology used for their production. Biofuels are also classified according to their source and types. They may be derived from forest, agriculture, fishery products or municipal wastes in addition to by-products and wastes originated from the agro-industry, food industry and food services. Biofuels can be solid (such as fuel wood, charcoal and wood pellets) or liquid (ethanol, biodiesel, and pyrolysis oils) or gaseous (biogas eg. methane) (Mehulkumar L et al 2013) .

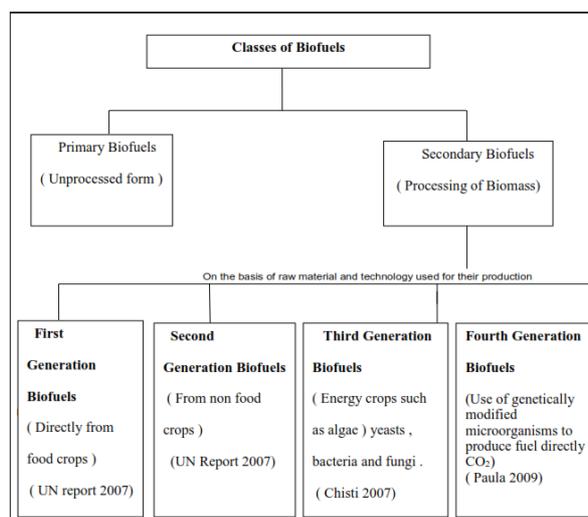


Figure. 2 Classes of Biofuels

Metabolic Engineering

The parts of metabolic pathways may exist in some microorganisms. Few have a complete pathways too or can synthesize the desired compounds efficiently but any pathway can be engineered into any host to produce the desired fuel. The challenges to face are to increase the titre, yield or productivity associated with biofuel production. In some cases the titre or productivity need to be increased by new biosynthesis routes 'tap' the metabolism ever more efficiently, particularly through the engineering of driving forces and utilization of cofactors generated during the light reactions of photosynthesis, resulting in higher product titers (James C et al 2016) .

Recently, a similar pathway for higher alcohol production was expressed in *E. coli* to yield six different straight chain and branched-chain alcohols and the same group has demonstrated production of 1.28 g/L of isopentanol by increasing the flux through the desired pathway (Connor MR, Liao JC 2008). Looking beyond natural pathways, hybrid processes that combine both biological and chemical production steps can also lead to new chemicals that could serve as biofuel (Roman-Leshkov Y 2007) . Some of the obstacles to achieve high yields are a result of the interdependence of metabolic networks, which are strongly influenced by the global levels of a handful of metabolites like ATP/ADP, NAD⁺/NADH, NADP⁺/NADPH, and acyl-CoAs. These central metabolites play an important role in regulating multiple pathways in the cell. The cell uses relative ratios of these metabolites to regulate different metabolic activities and ultimately results in the physiology of the cell. For example, the redox state of a cell is essentially determined by the relative ratio of NAD⁺ to NADH. The incorporation of new pathways for biofuel synthesis can destabilize the balance of these important metabolites, leading to the production of undesirable byproducts and a decrease in yield (Sung Kuk Lee et al 2008). One way to predict the impact of a new metabolic pathway on growth and product formation is through the use of metabolic models. Over the years, biochemical models of *E. coli* (Reed JL 2003) and *S. cerevisiae* (Forster J et al 2003) metabolism have become more sophisticated. The stoichiometric models can also be described by a set of determined equations using metabolic flux analysis (MFA), where the exchange fluxes are measured experimentally. MFA has been useful in studying the native metabolism of *E. coli* under different growth conditions (Kayser A et al 2005) and during recombinant protein production (Ozkan P et al 2005) . *In silico* genome-scale models have also been extended beyond gene deletion or over expression strategies to include gene insertion strategies for redox balancing. *In silico* gene insertion strategy was used to improve ethanol production and decrease the production of the byproducts like glycerol and xylitol (Bro C et al 2006). These techniques demonstrates the importance of monitoring and balancing the levels of various important metabolites in order to achieve optimal product titers. Consequently, metabolic engineering will play an important role in engineering efficient microbial pathways for the betterment of production of economically sustainable biofuels.

Solar Energy to Fuel

For successful advancement, one needs to come up with new to nature solutions and to construct synthetic living factories and designer microorganisms for efficient and direct conversion of solar energy to fuel. Photovoltaic-driven electrolysis is the more efficient process when measured on an annual basis, yet short-term yields for photosynthetic conversion under optimal conditions come within a factor of 2 or 3 of the photovoltaic benchmark (Robert E et al 2011). Photon-to-fuel conversion efficiency (PFCE) is used to describe the percentage of the energy of photons hitting the organisms and converted them in to chemical energy by photosynthetic light reactions ending up in the fuel. This terminology was chosen to make some rough comparisons between the efficiency of production of electricity by solar cells and the production efficiency of biofuels by a number of different production systems (Ingana's, O., and V. Sundstro'm. 2016) . Enhancement of photosynthesis concerns both the optimization of light-capturing antenna size and the electron transduction efficiency to maximally sustain the CO₂ fixation capacity (Maurino, V.G., and A.P.M. Weber. 2013) . Fourth generation biofuels take advantage of synthetic biology of algae and cyanobacteria (Berla, B.M et al 2013, Hays, S.G., and D.C. Ducat. 2015 and Scaife, M.A. et al 2015) . Introduction of various fermentative metabolism pathways to cyanobacteria cell by synthetic biology approaches has made it possible to produce biofuels directly from solar energy and Calvin–Benson cycle intermediates (Wijffels, R.H et al 2013, Halfmann, C et al 2014 and Savakis, P., and K.J. Hellingwerf. 2015) . While algal species are highly promising due to their ability to grow on cheap substrates and requires little bit CO₂ and sunlight but there is much less known about their genetic make-up and their slower growth than traditional model microbes which can be a major hindrance for rapid progress. However , there are many research groups and start-ups working on using algae to produce and even secrete, high-value fuel precursors (Liu X 2010) .

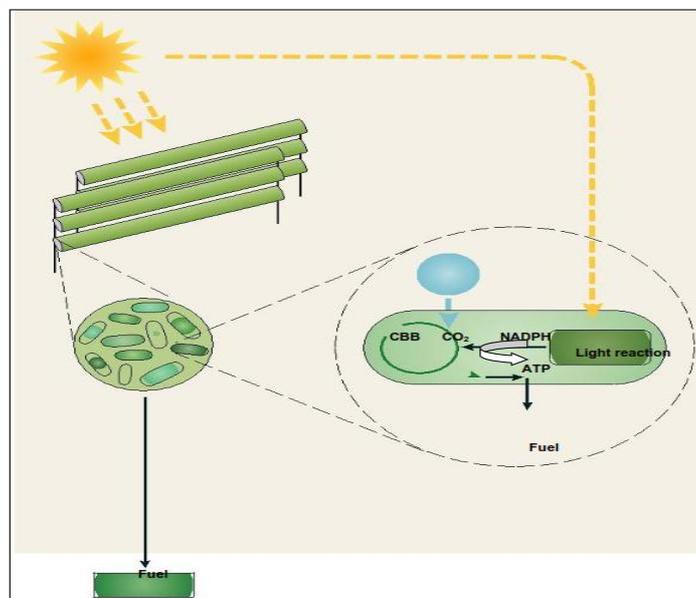


Figure. 4 Photosynthetic organisms, such as microalgae and cyanobacteria, can harness energy from sunlight to reduce CO₂ and convert it to liquid fuels

Activation of alternative pathway for glucose metabolism like PPP and EDP has resulted in an improved precursor and cofactor supply for isoprenoids production (Zhao, J et al 2013 and Liu, H 2014) . Generation of chimeric fusion proteins via connecting enzymes that are sequential in a pathway by a linker sequence has been shown as a promising tool to overcome the feedback inhibition of enzymes by intermediate (Wang, C et al 2011 and Sarria, S et al 2014) . Other approaches like (i) attaching proteins (Dueber, J.E 2009) , RNA (Delebecque, C.J et al 2011) and DNA (Conrado, R.J et al 2012) on a synthetic scaffolds (ii) localizing pathway intermediates into protein based synthetic compartments or bacterial micro compartments (Chen, A.H. and Silver, P.A. 2012 , Lee, Het al 2012 and Woolston, B.M et al 2013) (iii) tagging proteins with localization signals (Martin, W 2010) have disclosed to reduce metabolic load and improve productivity. RNA- sequencing technologies have helped to identify genes that are involved in the accumulation of triacyl- glycerol (TAG) under conditions of nitrogen deprivation (Rodolfi, L. et al 2009); providing potential targets for the engineering of strains with a high level of lipid production. Gene-editing techniques have also altered the engineering of cyanobacteria which are genetically more tractable than eukaryotic microalgae and therefore more versa-tile for the direct production of fuels and chemicals. For example, introducing exogenous biosynthetic pathways into *Synechococcus elongatus* PCC 7942 or *Synechocystis* sp. PCC 6803 has led to the production of various compounds directly from CO₂ (Angermayr, S. A et al 2015) . While in case of hyperthermophilic archae on *Pyrococcus furiosus* is an obligate heterotroph that grows optimally (Topt) at 100°C by fermenting sugars to hydrogen, carbon dioxide and acetate (Fiala G, Stetter KO 1986) .It cannot use carbon dioxide as its sole carbon source. A novel means of metabolic control was recently reported in *P. furiosus* that exploited the difference in the temperature dependence of the host's metabolism and the inserted foreign synthetic pathway (Basen M et al 2012) . Electrofuels microbes are derived from exotic bacteria that live underground or in other places (such as geothermal springs) where photosynthesis doesn't occurs. In the wild, these organisms survive by "eating" electrons derived from minerals in the surrounding soil. But in the lab, their genes are transferred to other bacteria that can more easily be grown in vats hooked up to a power grid that can provide the needed electricity (web 2013) .

Conclusions

Increasing energy demand and fuel costs resulted in increased attention to explore alternatives for fossil fuels. For fulfilling the demands of ever increasing population from developing countries one needs to explore and develop the technology for biofuel production which is environmental friendly and cost effective. We are armed with better tool to engineer the microbes for better yield with synthetic biology and Photovoltaic approach. This article covers classes and revolution of biofuels production using metabolic engineering, synthetic biology and photovoltaics approach including challenges and hurdles. Techniques and approaches covered in this article will surely play important roles in its evolution and gives the snapshot for biofuel production with updated technology.

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