

Approaches for Alternative Biofuels for Global Fuel Demand: A Review

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ABSTRACT

The increasing global fuel demand is a great challenge as reduction in fossil fuel stock at an alarming rate is a matter of great concern and has led to search for an alternative fuel that should be clean, ecofriendly and sustainable. At this juncture the global scientific community should focus on discovering and developing modified energy crops with enhance properties of producing biofuels. The plant fatty acid seeds containing triacylglycerols has emerged as a best alternative with potentiality to be used as a fuel. Various fatty acid seeds containing triacylglycerols can be synthesise into useful fuel using modern scientific tools and techniques through biotechnological principles. A series of methods and mechanism are formulated for converting viscous liquid into fatty acid esters and enhancing the fuel property in the plant seeds. The various methods for biofuel production, along with the modern and modified biotechnological production techniques and their relevancy are reviewed. The challenges and limitations witnessed during this operation are also discussed.

Keywords-alternative fuel; biotechnology applications; fatty acid seeds, triacylglycerols

INTRODUCTION

Biodiesel (fatty acid methyl esters), which is derived from triglycerides, has attracted considerable attention during the past decade as a renewable, biodegradable, and nontoxic fuel (Fukuda et al, 2001) and has become an attractive diesel fuel substitute due to its environmental benefits (Marchetti et al, 2008). It is gaining more and more importance as an attractive fuel due to the depleting fossil fuel resources (Meher et al, 2006). Biodiesel production is a very modern and technological area for researchers due to the relevance that it is winning everyday because of the increase in the petroleum price and the environmental advantages (Marchetti et al, 2007). The global

market for biodiesel has been growing rapidly during the past few years, and it is poised for explosive growth in the next years (Deng et al, 2009). Research and development efforts for biofuel production are targeted at converting plant biomass into renewable liquid fuels. Researchers are expanding the genetic and genomic resources available for crop improvement, elucidating lipid metabolism to facilitate manipulation of fatty acid biosynthetic pathways and studying how plant cell walls are synthesized and assembled. This knowledge will be used to produce the next generation of biofuel crops by increasing fatty acid content and by optimizing the hydrolysis of plant cell walls to



release fermentable sugars (Vega-Sánchez and Ronald, 2010).

PLANT OILS: ALTERNATIVES OF CONVENTIONAL DIESEL

Triacylglycerols produced by plants are one of the most energy-rich and abundant forms of reduced carbon available from nature. Given their chemical similarities, plant oils represent a logical substitute for conventional diesel, a non-renewable energy source. However, as plant oils are too viscous for use in modern diesel engines, they are converted to fatty acid esters. The resulting fuel is commonly referred to as biodiesel, and offers many advantages over conventional diesel. Chief among these is that biodiesel is derived from renewable sources. In addition, the production and subsequent consumption of biodiesel results in less greenhouse gas emission compared to conventional diesel (Durrettet al, 2008).

Chemical structure of triacylglycerols

Triglycerides or triacylglycerols have a glycerin backbone joined by ester linkages to three fatty acid chains. The chemical structure shows the different areas within a typical oil (triglyceride) molecule with one of the three ester linkages circled. Triacylglycerol is composed of three fatty acyl groups esterified to a glycerol backbone at the *sn*-1, *sn*-2 and *sn*-3 positions. The fatty acid portions may vary in length between C12 and C18. In higher plants, triacylglycerol is the predominant component of the oil of the seeds or fruits of oleaginous plants and primarily serves as an energy store to support the growth of the young seedling during the early stages of germination. Triacylglycerol is also an important part of our bio-economy, providing a source of highly reduced carbon for both food and non-food applications,

such as supplying a feedstock for the production of petrochemical alternatives.

Synthesis of triacylglycerols in plants

Chemically biodiesel is monoalkyl esters of long chain fatty acids derived from renewable feed stock like vegetable oils and animal fats (Meher et al, 2006). There should be an increased understanding of how plants synthesize fatty acids and triacylglycerols which will ultimately allow the development of novel energy crops. For example, knowledge of the regulation of oil synthesis has suggested ways to produce triacylglycerols in abundant non-seed tissues (Durrettet al, 2008).

The biosynthesis of triacylglycerol occurs in the endoplasmic reticulum (ER) and involves acyl-editing of fatty acyl chains within the nitrogenous phospholipids of the ER. Depending on the plant species, particular reactions of triacylglycerol assembly and acyl-editing may be catalyzed by one or more forms of an enzyme. In addition, in some cases, variants or allelic variants of the same isoenzyme have been identified. Currently, there are no three-dimensional structures available for any of these enzymes. Triacylglycerol droplets accumulate in the outer leaflet of the ER and eventually 'bud off' from the ER to form oil bodies ranging from 0.5 to 2.5 micrometers in diameter which are surrounded by a monolayer of phospholipid, with amphiphilic oil body proteins embedded in the triacylglycerol and phospholipid layer. Although triacylglycerol accumulates to high levels in the seeds and fruits of oleaginous plants, triacylglycerol is also known to collect in other plant parts including pollen and vegetative tissue (Weselake et al, 2010).



BIODIESEL FUEL PRODUCTION METHODS

There are four primary ways to make biodiesel, direct use and blending, microemulsions, thermal cracking (pyrolysis) and transesterification (Ma and Hanna, 1999). Among these transesterification using alkali-catalysis gives high levels of conversion of triglycerides to their corresponding methyl esters in short reaction times. Transesterification is the process of transforming one type of ester into another type of ester. The reaction is catalyzed by the presence of the strong base, NaOH. In the first step of the reaction, the NaOH reacts with methanol in an acid base reaction. The products of this first step of the reaction are a very strong base, sodium methoxide, and water. In the second step, the sodium methoxide breaks the glycerine section from the fatty acid section. The separation of the glycerine portion leads to the formation of three methyl esters (the biodiesel) and glycerol. The NaOH is regenerated as a product in the reaction. The biodiesel and glycerol are immiscible and will separate to form two layers. The glycerol layer will also contain NaOH and excess methanol. The separation of the biodiesel and glycerol layer is fortuitous in that we can easily separate and isolate our biodiesel product from the remaining product mixture. The process of transesterification is affected by the mode of reaction condition, molar ratio of alcohol to oil, type of alcohol, type and amount of catalysts, reaction time and temperature and purity of reactants ((Ma and Hanna, 1999, Meher et al, 2006).

Biodiesel is a fuel, which means that it produces energy through combustion with oxygen (as does regular diesel). The combustion reaction each carbon contained in biodiesel is converted to

carbon dioxide. As each carbon is oxidized to carbon dioxide it will release about 850 kJ per mole. The more carbons, the more energy produced upon combustion. Vegetable oil itself is also a fuel and can undergo combustion with oxygen. So the vegetable oil (triglycerides) is converted to the methyl esters as the triglycerides shows viscosity i.e., the internal friction or stickiness of a liquid. A viscous material is 'thicker' and does not flow quickly. The viscosity of vegetable oil will present a problem in that its stickier nature will prevent it from flowing easily through the fuel pump systems of an engine. Since viscosity is generally inversely proportional to temperature, at lower temperatures the viscosity problem is enhanced. Unlike the triglycerides, methyl esters (biodiesel) are less viscous and will easily flow through the fuel system of an engine, though there is some controversy over the type and percentage of biodiesel that can be used in a standard diesel engine due to issues with gelling of the fuel.

PLANT BIOTECHNOLOGY AND BIODIESEL PRODUCTION

The generation of transgenic lines of plant seeds with high oleic acid content represents one way in which plant biotechnology has already contributed to the improvement of biodiesel (Durrett et al, 2008). In order to meet global production needs for lowcost, energy-dense lipids such as triacylglycerol (TAG) high biomass crops are engineered for increased lipid content in vegetative tissues such as leaves (Chapman et al., 2013; Dyer et al., 2012; Troncoso-Ponce et al., 2013). The biotechnological techniques are applied for accumulation of TAG to levels above 10% on a dry weight basis in a dedicated energy crop, which has been found to increase the energy

yield in such a crop by at least 30% (Ohlrogge and Chapman, 2011).

Current metabolic engineering attempts, however, have only achieved low levels of storage lipid accumulation in nonseed plant tissues far below this benchmark. In order to achieve industrially relevant levels of storage lipids in vegetative tissues, likely requires optimizing the flux of carbon into TAG on multiple metabolic levels including fatty acid synthesis ('Push'), TAG assembly ('Pull') and lipid turnover ('Protect'). Consequently, some groups have attempted to further increase TAG accumulation in nonseed tissues by modifying the expression of gene pairs. Oleosins typically coat and stabilize oil bodies in oilseeds but are normally not expressed in leaf tissues. The improved stability of the lipid droplets and a remarkable increase in biomass are reported. An alternative approach consisting of diverting the flux of carbon from starch-to-lipid biosynthesis by silencing ADP-glucose-pyrophosphorylase (AGPase), was also noted. TAG biosynthesis might only be increased during part of the circadian cycle, thereby reducing possible unwanted metabolic impacts on leaf function and plant development that might be the result of a restriction in photosynthate supply. The effect of the combined transient overexpression of the transcription factor and genes shows a significant synergistic effect on leaf TAG content. The stable expression of this particular gene combination might result in even greater TAG accumulation due to a longer gene expression window (Vanhercke et al., 2014).

It will be worthwhile to determine whether high TAG levels can be achieved simultaneously in all

vegetative tissues, thereby optimally using the entire vegetative biomass for renewable production of storage lipids. The demonstrated engineered high levels of storage oils in vegetative tissues should also enable and motivate further studies of TAG biosynthesis and turnover in non-seed tissues and of the intricate relationship with other carbon pools such as starch. Due to the myriad of applications of a high vegetative oil production platform, including biofuels, food and oleochemical feedstocks, we are confident that the global plant lipid research community will rapidly increase the level of TAG in several application species with fatty acid profiles tailored according to a variety of specific needs (Vanhercke et al., 2014). Importantly, although plant biotechnology will be key to the successful generation of energy crops, it should go hand in hand with breeding efforts targeted at maintaining or enhancing the important agronomic traits that made the biofuel producing plants so attractive for biofuel production to begin with, namely resistance to abiotic and biotic factors, low fertilization requirements and perennial life cycle (Vega-Sánchez and Ronald, 2010).

EVALUATION OF MODIFIED BIOFUEL PRODUCTION TECHNIQUES

Transesterification process has therefore been widely utilized for biodiesel fuel production in a number of countries. Recently, enzymatic transesterification using lipase has become more attractive for biodiesel fuel production, since the glycerol produced as a by-product can easily be recovered and the purification of fatty methyl esters is simple to accomplish. The main hurdle to the commercialization of this system is the cost of lipase production. As a means of reducing

the cost, the use of whole cell biocatalysts immobilized within biomass support particles is significantly advantageous since immobilization can be achieved spontaneously during batch cultivation, and in addition, no purification is necessary. The lipase production cost can be further lowered using genetic engineering technology, such as by developing lipases with high levels of expression and/or stability towards methanol. Hence, whole cell biocatalysts appear to have great potential for industrial application (Fukuda et al, 2001).

On the other hand, homogeneous acidic catalysts are being used for exactly such feedstocks. Both acid and basic homogeneous catalyzed processes require downstream purification equipment to neutralize the catalyst and to purify the biodiesel as well as the glycerol. Recent studies have been conducted to employ heterogeneous catalysts, such as acidic or basic solid resins, or immobilized lipases. These catalysts will allow the use of different feedstocks that will permit operation at lower investment costs and will require less downstream process equipment (Marchetti et al, 2008). The used cooking oils are used as raw material, adaption of continuous transesterification process and recovery of high quality glycerol from biodiesel by-product (glycerol) are primary options to be considered to lower the cost of biodiesel (Ma and Hanna, 1999). Additionally, biodiesel has poor cold-temperature performance and low oxidative stability. Improving the fuel characteristics of biodiesel can be achieved by altering the fatty acid composition (Durrett et al, 2008).

CHALLENGES AND LIMITATIONS FOR WIDESPREAD ADOPTION OF BIODIESEL

The widespread adoption of biodiesel faces a number of challenges. The biggest of these is a limited supply of biodiesel feedstocks. Thus, plant oil production needs to be greatly increased for biodiesel to replace a major proportion of the current and future fuel needs of the world. The biodiesel has poor cold-temperature performance and low oxidative stability thus there is a need of improving the fuel characteristics of biodiesel which can be achieved by altering the fatty acid composition (Durrett et al, 2008). The cost of biodiesel, however, is the main hurdle to commercialization of the product (Ma and Hanna, 1999). This remains the main problem in making it competitive in the fuel market either as a blend or as a neat fuel. However, more than 80% of the production cost is associated with the feedstock itself and consequently, efforts are focused on developing technologies capable of using lower-cost feedstocks, such as recycled cooking oils and wastes from animal or vegetable oil processing operations. The main issue with spent oils is the high level of free fatty acids found in the recycled materials. The conventional technology employs sodium methoxide as a homogeneous base catalyst for the transesterification reaction and illustrates the drawbacks in working with feedstocks that contain high levels of free fatty acids.

A potential approach for increasing the energy density of plant biomass by engineering plants to accumulate TAGs in vegetative tissues such as leaves stems and roots which is an alternative for the production of biodiesel should be preferred. However, in the case of bioenergy trees the biotechnological techniques should be employed to accumulate oils in stems which will have an

increased impact on bioenergy production. The accumulation of TAGs in plant tissues by blocking the sucrose to starch conversion in chloroplasts or blocking the degradation of TAGs in peroxisomes has also got success in recent years. Future research for improving the generation of biofuels should focus on improving lipases for efficient oil transesterification. However, the lack of oil feedstocks limits the large-scale development of biodiesel to a large extent. Therefore the global scientific community should pay attention for more promising technology for biodiesel production.

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