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Research review paper

# Designing the perfect plant feedstock for biofuel production: Using the whole buffalo to diversify fuels and products

B.L. Joyce [a], C.N. Stewart Jr. [a,b,*]

[a] Department of Plant Sciences, 2431 Joe Johnson Drive, University of Tennessee, Tennessee 37996, USA
[b] BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37931 USA

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ABSTRACT

Petroleum-derived liquid fuels and commodities play a part in nearly every aspect of modern daily life. However, dependence on this one natural resource to maintain modern amenities has caused negative environmental and geopolitical ramifications. In an effort to replace petroleum, technologies to synthesize liquid fuels and other commodities from renewable biomass are being developed. Current technologies, however, only use a portion of plant biomass feedstocks for fuel and useful products. "Using the whole feedstock buffalo" or optimally using all portions and biochemicals present in renewable biomass will enhance the economic and environmental feasibility of biofuels and coproducts. To accomplish this optimization, greater understanding of the relationship between liquid fuel and bioproduct properties and plant chemistries is needed. Liquid fuel properties and how they relate to biochemistry and petrochemistry are discussed. Enhanced biofuel yields and high-value commodities from biomass are needed to sustainably replace petroleum-based products. Several metabolic engineering strategies are discussed. We will describe paths of possible fuel and product diversification using dedicated lignocellulosic biomass (e.g., switchgrass).


**Contents**



## 1. Introduction

Modern, industrialized society relies on a single natural resource to provide a plethora of commodities and conveniences that would be hard to envision living without: petroleum. Petroleum not only provides liquid transportation fuels, but also provides the asphalt which literally paves the way for transportation. Petroleum provides heating fuels, plastics and other materials which have revolutionized everything from how we package and store food to modern medical

*Abbreviations:* SSF, simultaneous saccharification and fermentation; FAME, fatty acid methyl ester; FAEE, fatty acid ethyl ester; PHA, polyhydroxyalkanoates; PHB, poly-3-hydroxybutyrate; TAL, tyrosine ammonia-lyase; HCT, hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase; DGAT, diacylglycerol acyltransferase; LEC, leafy cotyledon.

* Corresponding author at: 2431 Joe Johnson Drive, University of Tennesse, Knoxville, TN 37996–4561. Tel. +1 865 974 7324; fax: +1 865 946 1989.
*E-mail address:* nealstewart@utk.edu (C.N. Stewart).

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products (Thompson et al., 2009). Petroleum has in some way contributed to nearly every aspect of modern daily life, but the end of petroleum is in sight.

But for every positive benefit that petroleum has provided there seems to be a negative environmental ramification. The Deepwater Horizon oil spill released 4 million barrels of oil into the Gulf of Mexico which has had a series of ecological and economic impacts on the states and countries lining the Gulf (Camilli et al., 2010). Extensive use of asphalt have created a phenomenon known as 'urban heat islands' which increases energy consumption and can increase mortality rates in urban centers (Rizwan et al., 2008). Emissions from combustion engines have led to debate and growing concern over air quality and greenhouse effects. Plastics make up 10% of human wastes, do not readily degrade, and when they do they release toxic chemicals that have started to bioaccumulate across the globe (Thompson et al., 2009). There have also been negative geopolitical ramifications associated with petroleum production and consumption. Included in the list is war, internal friction within countries, economic and political instability, and increasing disparity between rich and poor countries. Taken together these considerations have led researchers to investigate a number of technologies to replace petroleum-derived commodities with renewable, ubiquitous, and more environmentally benign substitutes. Replacing petroleum commodities with an inexpensive, renewable resource that can be produced in any country in the world would lead to a second green revolution for human needs going beyond food (Mooney, 2009).

Bioplastics derived from natural polymers are renewable and biodegradable (Mooney, 2009; Suriyamongkol et al., 2007). Of specific interest, biofuel research has taken aim at replacing petroleum liquid fuels with chemicals derived from crop and forest residues, algae, and bioderived waste materials. There have been a number of policies and incentives directed at developing both ethanol (Hoekman, 2009; Martin, 2010) and biodiesel (Hoekman et al., 2009) into mature cost-effective technologies. However, current biofuels are not ideal liquid fuels when characteristics like fuel properties and compatibility with existing infrastructure are considered. These benefits and drawbacks will be further discussed in Section 2.1. Plant biotechnology and microbial biotechnology have been proven to be useful tools in improving biomass processing and biorefinery product yields (Hermann and Patel, 2007; Octave and Thomas, 2009). Biocatalyst reactions, or reactions driven by enzymes, have advantages over organic chemistry synthesis, e.g. the ability to produce complex molecules efficiently (Wohlgemuth, 2009). Although the use of biocatalysis of chemicals on large scale has been limited, biotechnology and bioprocessing have been applied extensively to biofuel production, which will be discussed in Section 3. The reasons why biofuels have become an attractive solution to replacing petroleum-derived liquid fuels has been addressed in a number of reviews, and as such is beyond the scope of this manuscript, e.g. Hoekman, 2009. However, it is important to briefly discuss how first generation (food crop-derived) and second generation (non-food crop-derived) biofuels developed to better understand next generation biofuels and bioproducts.

In 2007, the U.S. Energy Independence and Security Act set incentives and a goal of 144 billion liters of biofuels per year by 2022 (Martin, 2010). Industry and researchers turned to available technologies in an attempt to begin to displace petroleum fuels immediately. In the US, ethanol was first derived from fermented starch (usually maize grain); biodiesel was derived from alkyl esters of cooking or waste oils. Starch and plant oils are feedstocks easily accessible and have liquid fuel synthesis technologies already well developed (Octave and Thomas, 2009); in the case of ethanol fermentation, humans have been practicing it for millennia. But these technologies led to a now famous public outcry against using food sources to produce fuels. The outcry resulted from food prices that increased 4.0% in 2007 and 5.5% in 2008 compared to a 2.4% increase in 2006 and 2005 (Martin, 2010). In reality, the higher food prices were a result of several factors with corn-based ethanol production accounting for only about a fifth of the total food price increase of 4.0 and 5.5% (Martin, 2010). Despite this, biofuel research shifted more heavily to non-food sources such as corn stover and dedicated biofuel crops such as poplar, switchgrass, and algae.

## 2. Replacing petroleum commodities: can we grow barrels of oil?

The ultimate goal of biofuels is to completely replace petroleum-derived liquid fuels, especially for the transportation sector. But biomass, like a barrel of oil, contains a diverse array of chemicals that could be used to create many different commodities in addition to liquid fuels. Indeed, fuel could be the essential loss leader in the emerging bioeconomy (Bozell, 2008). Petroleum itself is formed from organic matter such as marine algae and plants heated to specific temperatures in the Earth's crust on geologic time scales. The formation of petroleum occurs throughout the world and the chemicals that are formed differ based on different locations and different source rock (Speight, 1999). Petroleum is so chemically complex and variable between each deposit that it has been traditionally characterized by bulk properties like distillation ranges and total atomic percentage. In fact, it was not until recently that individual chemicals present in petroleum could be identified using high resolution mass spectrometry (Marshall and Rodgers, 2008). The chemical complexity of petroleum has led to the petroleum industry adopting a number of technologies, e.g. catalytic reforming, hydrotreating, etc., to separate, refine and alter chemical fractions for specific uses (Matishev, 1994).

By comparison, biomass is an immature feedstock compared with petroleum that needs to be converted and refined into chemicals useful for commodities. In the current biofuel industry, chemically complex plant biomass is separated, thermally cracked or degraded by enzymes, and then converted into products using chemical synthesis or biological conversion. The key is the efficient conversion of biomass into petroleum-like chemicals on a biological timescale (seconds to hours) rather than a geologic timescale (millennia). The main factor that distinguishes petroleum from biomass is the use of biotechnology to fundamentally alter enzymes present in biomass; essentially, biotechnology enables researchers to engineer and fine-tune barrels of renewable (biomass-derived) petroleum. To put the concept into petroleum terminology, biotechnology could be considered *in vivo* refining, and can occur in plant biomass, microbes used to ferment the biomass, or a combination of both. A significant amount of work has gone into altering fermentation products in microbes, and several comprehensive reviews are available (Lee et al., 2008; Liu and Khosla, 2010; Peralta-Yahya and Keasling, 2010). Currently suspension cells, micropropagated plantlets, and hairy root culture are the main mechanisms for industrial scale *in planta* production of high-value biochemicals, but each of these methods has major drawbacks that limit their wide commercial success (Weathers et al., 2010). Microbial fermentation of products has been thoroughly studied and has several advantages over *in planta* synthesis of metabolites such as rapid screening on culture chips, short life cycles, ease of engineering resulting from relatively simple metabolic pathways, and more sequence data available (Wohlgemuth, 2009). As dedicated biofuel crops become more commonplace, however, *in planta* synthesis of biochemical products offers several advantages such as simple extraction and separation to yield products, and direct (efficient) synthesis of hydrocarbons and high-value commodities using low-cost solar energy. Synthesis of biofuels and chemical commodities in traditional agricultural crops would allow countries without extensive infrastructure to produce modern commodities and could increase overall yields of biofuels by reducing loss of fixed carbon resulting from conversion. Cyanobacterial or algal production of biofuels might likely be the best combination of microbial and plant production systems, but there are still significant barriers to these technologies and their use on a sustainable industrial scale remains in the long-term (Wijffels and Barbosa, 2010).