A Solution-Focused Comparative Risk Assessment of Conventional and Emerging Synthetic Biology Technologies for Fuel Ethanol

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Introduction

Global energy demand is increasing due to global development initiatives and steady population growth. The US Energy Information Administration's International Energy Outlook 2017 (U.S. EIA 2017c) projects that the world energy consumption will raise from approximately 575 quadrillion Btu in 2015 to 736 quadrillion Btu by 2040—an increase of 28% (U.S. EIA 2017c). Fossil fuels, such as petroleum and natural gas, serve as the leading energy sources for various sectors, such as transportation. However, the International Energy Agency (IEA) forecasts that biofuel production will increase by 15% over the next 5 years to reach approximately 42.6 billion gallons (IEA 2018). Various types of renewable fuels or fossil fuel additives are being researched and developed as complements or supplements to fossil fuels. Ethanol, or ethyl alcohol, is one such additive, particularly for motor fuel in the United States and Brazil. Fuel ethanol has been proprosed to offset dependence on petroleum, thereby reducing greenhouse gas emissions by up to 43% relative to gasoline (Flugge et al. 2017). Additionally, as advanced ethanol production processes are less sensitive to the vagaries of geography, as will be discussed later in

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this chapter, countries can produce it domestically rather than having to rely on the geopolitics associated with the world petroleum market.

Ethanol is directly blended with petroleum to comprise approximately 10% the volume of each gallon of gasoline consumed through US gas stations (US EIA 2016). In 2017, approximately 27 billion gallons of ethanol was produced internationally, with a projected growth rate of 2% each year. It is predicted that fuel ethanol will account for approximately two-thirds of overall biofuel production growth and that by 2023, the annual output of ethanol will be 31 billion gallons internationally (IEA 2018).

Ethanol is currently produced by converting a variety of feedstock sources into useful sugars. While existing ethanol production has been derived primarily from corn and sugarcane feedstocks, advanced production methods have the potential to use various species of algae to produce an algal oil substitute. Strains of naturally occurring algae are capable of yielding such algal oil in limited quantities, but innovative technologies utilizing synthetic biology are being considered to improve the production process. Synthetic biologists are interested in developing strains of engineered algae in controlled environments to produce ethanol with more efficient and renewable ethanol yield rates.

Yet, engineered algal ethanol imposes unique risks, benefits, and other implications. As an emerging technology, synthetic biology processes introduce issues of uncertainty and complexity that derive from the novelty of the technology. Further, there are limited data pertaining to these emerging technologies, which makes it difficult to precisely quantify the risks and to subsequently improve best practices. However, these same emerging technologies can provide significant benefits to human and environmental health, such as improved air quality relative to fossil fuels. Understanding that current fossil fuel and conventional ethanol production entail risks of their own, it is crucial to compare energy sources based on both risk and potential benefits. Rather than asking whether engineered algal ethanol is efficacious and "safe enough" for deployment in its own right, a comparative approach is critical to assess various attributes of the emerging energy source against the risks and benefits of the best conventional solutions to meet national and international energy demand. Conventional quantitative risk assessments (QRA) measure quantitative data pertaining to an alternative's risks; as synthetic biology is emerging and field use is limited, the critical quantitative data are limited, and a modified approach to emerging technology risk assessment is necessary (Malloy et al. 2016; Linkov et al. 2018).

A solution-focused risk assessment (SFRA) (Finkel 2011; Finkel et al. 2018) is one such approach that can qualitatively and quantitatively evaluate synthetically engineered algal ethanol relative to conventional competitors. In general, SFRA tries to transcend traditional risk assessment questions ("is it safe enough?" or "what level of exposure yields an acceptably low risk?" to instead require risk assessors and decision-makers to collaborate from the earliest point and address broader questions of which of several competing technologies best fulfills a given human need (considering both risk reduction benefits and new downside risks) (see Finkel et al. 2018). Specifically, this chapter introduces an SFRA that assesses economic and social implications, sustainability, environmental implications, and risk considerations. These considerations will be compared across corn, sugarcane, and algal ethanol (natural and engineered) production processes. The trade-offs between risks and benefits are evaluated. The benefits of the various ethanol sources are weighed against their potential risks in order to conceptualize the net risk reduction for each ethanol source, relative to the others. Because the environmental and human health benefits of ethanol fuel, once it is produced, do not depend on the means by which it was generated, we only need to compare the downside risks of the various technologies (in many other cases, the products derived via synthetic biology approaches differ from the conventional product it seeks to displace, and so the risk reduction benefits may differ and need to be accounted for). Therefore, this SFRA approach compares whether the risks of conventional ethanol production (e.g., land use requirements for conventional ethanol sources) outweigh the novel risks of emerging ethanol production methods.

In this chapter, a general framework for an SFRA is laid out with recommendations for how to interpret input and outcome measures and for how future research could build from this framework. The SFRA presented here provides a framework to consider the risks of each feedstock option by using ranges of measures found in existing literature. The SFRA approach puts problem *decisions* at the forefront of risk reduction; in this case, what bioethanol feedstock options minimize adverse economic and environmental implications and risks. Rather than focusing on estimating an acceptable level of risk, SFRA aims to identify which decision or alternative has the greater net risk reduction. Ideally, this comparative approach allows for the benefits of certain, perhaps advanced or novel, alternatives to be realized in comparison to status quo technologies (e.g., petroleum production and consumption) should the advanced alternatives provide net risk reduction. The net risk reduction of alternatives is compared across four factors: sustainability, environmental implications, social and economic implications, novel risks. Future sensitivity analyses performed on these metrics could assess the decision thresholds across the four factors while fine-tuning the choice among technologies within specific locations and economies and across uncertain parameters. This is particularly crucial for synthetically engineered algal ethanol, for which limited public empirical data exist. The less predictable, novel risks associated with synthetically engineered algae are discussed with guidance on how to overcome the ambiguity associated with incorporating and comparing "known" and "unknown" risks.

Background: Development of Fuel Ethanol

Before discussing the current sources of ethanol feedstock and their production processes, it is necessary to review the history of ethanol development and eventual commercialization. The production of the various types of ethanol dates to the Neolithic Period (4500–2000 BC) when sugar was fermented into ethanol for alcohol production (Roach 2005). Early ethanol production centered on the distillation of wine and spirits for alcoholic beverages, where these ethanol precursors were derived from grapes, rice, and other agricultural plants. Ethanol production for fuel use took off in the early nineteenth century, when Swiss chemist Nicolas-Théodore de Saussure determined ethanol's chemical formula in 1807 (de Saussure 1807). This formula served as the basis for early synthetic ethanol production from ethylene or coal gas. The early modern use of ethanol centered on lamp fuel in the mid-nineteenth century, although various tariffs and taxes on ethanol use prohibited large-scale commercialization in the United States (Solomon et al. 2007; Tyner 2008; Campbell et al. 2008; Segall and Artz 2007). These efforts were driven by the belief that ethanol fuel could serve as a more efficient and cleaner burning alternative to traditional oils or coal, which had been widely used throughout the early Industrial Revolution.

The first modern and widespread commercial application of ethanol as automobile fuel for an internal combustion engine dates to early vehicles in the 1910s and 1920s (DiPardo 2000). These vehicles established the framework for future gasoline-ethanol blends, where Ford's early automobiles were able to operate on either gasoline or ethanol (DiPardo 2000). Today, virtually all of the commercial fuel ethanol production worldwide is produced by private companies in the United States, including Valero, Poet, Flint Hills Resources LP, Green Plains Renewable Energy, and ADM, by the state-run Brazilian company Petrobras, or external companies such as Raizen (Lovins 2005; Renewable Fuels Association 2016a, b). By 2011, companies (state-run or fully private) were responsible for approximately 87% of worldwide fuel ethanol production, or over 19 billion gallons of ethanol (Renewable Fuels Association 2011; Renewable Fuels Association 2012).

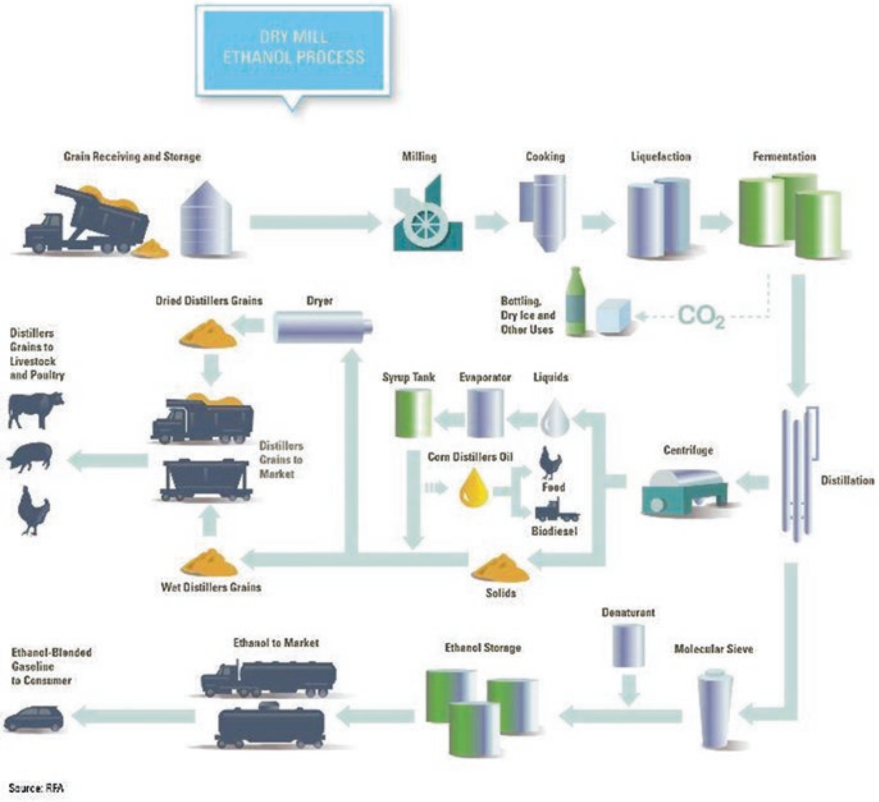
Similar to their American counterparts at Ford, Brazil's conversion of sugarcane into ethanol began in the late 1920s with the introduction of automobiles to the country (Valdes 2011). Ethanol production from sugarcane grew dramatically during World War II as oil shortages arose, which led the Brazilian government to mandate 50% ethanol fuel blends (Kovarik 2008). While sugarcane ethanol production declined post-war in the midst of cheap gasoline, it increased again during the oil crises in the 1970s and 1980s. Due to these oil crises, the Brazilian government has since directly funded private and state-run ethanol companies in an effort to phase out dependency on foreign fossil fuels in favor of domestic biofuels like sugarcane ethanol (Bastos 2007). The Brazilian national government formalized their efforts to promote sugarcane ethanol production in Programa Nacional do Álcool, or the National Alcohol Program, started in 1975 (Bastos 2007).

Conventional Ethanol Production Processes

Conventional ethanol production requires a crop or biomaterial to be transformed and manipulated from its native state into a liquid. Specifically, this occurs in different physical and chemical processes, including biomaterial growth, collection, dehydration, and fermentation. The dehydration and fermentation stages are used to convert the raw biomass into ethanol by removing excess water from the biomass and chemically converting plant sugars into energy.

The conversion of biomass to ethanol is a multiphase process that involves significant fuel expenditure (Pimental 2005). These steps needed to convert a crop to ethanol or biodiesel may differ based on the particular crop or biomatter used for fuel conversion yet generally follow the sequence of growth, collection, dehydration, and fermentation to yield ethanol. Each stage of the generic life cycle is further described below (Von Blottnitz and Curran 2007) (Fig. 1).

The first stage in the generic ethanol production process includes the growth of the feedstock for eventual conversion into ethanol fuel. This crop growth does not substantially differ from how the crops are grown for food. Additionally, crop growth can be multipurpose, where ripened crops may be used for ethanol or food, depending on the stakeholder's interests. With respect to corn, approximately 40% of all corn grown in the United States, or roughly 130 million tons, will be used for corn ethanol (Mumm et al. 2014). The timeframe for growth will differ based upon the crop grown and seasonality, with corn being cold-intolerant and planted in the



Source: RFA

Fig. 1 Generic ethanol fuel life cycle. (Source: Renewable Fuels Association (2016c)) summer months (Pollack 2011). Sugarcane is generally only grown in warm temperate to tropical regions in South America and South Asia, with 75 tons of raw sugarcane produced annually in Brazil per hectare of cultivation (Da Rosa 2012). This makes sugarcane production in Brazil an economically important industry, with benefits for both improved energy efficiency and a significant source of employment for locals.

Once grown, the crops are harvested and organized based upon their intended purpose (ethanol, food, etc.). For corn and sugarcane, each individual ear is harvested by hand or by a mechanical picker and is stored in bins that are designed to keep moisture levels low via “grain dryers” (Van Devender 2011). For sugarcane, each plant is capable of multiple harvests, so collection methods are careful not to damage the sugar-producing plant. According to Rakkiyappan et al. (2009), mechanical methods of collecting sugarcane are capable of collecting approximately 100 tons per hour, while a seasoned sugarcane harvester can cut roughly 500 kilograms per hour, where by-hand harvesting accounts for more than half of sugarcane collection annually, ensuring a steady demand for physical labor. Regardless of the method used, the collected sugarcane must be processed quickly once harvested, as it almost immediately begins to lose its sugar content once harvested (Rakkiyappan et al. 2009).

After harvesting, crops intended as biomass for ethanol are dehydrated and distilled to prepare them for eventual fermentation. Dehydration involves the drying of crops and is generally conducted using one of three processes, including azeotropic distillation, extractive distillation, and molecular sieves (Kumar et al. 2010; Rouquerol et al. 1994). Overall, the general purpose of each of these methods is to quickly remove any retained liquid from the feedstock. This prevents the material from spoiling during the ethanol production process and prepares the feedstock for its eventual fermentation.

The last step in ethanol creation is fermentation, through which sugars such as fructose, sucrose, and glucose are converted into energy (Stryer 1975). More specifically, the conversion of sugars into energy produces ethanol and carbon dioxide as waste material, where the ethanol may be sequestered for eventual use as fuel (Stryer 1975). Once produced, ethanol is then blended with gasoline and burned—normally for an internal combustion engine. While not directly covering any stage of ethanol production, the “burning” phase is reviewed in order to determine the environmental impact associated with burning ethanol and releasing toxic material into the environment.

Advanced Ethanol Production Processes

Within the United States and abroad, conventional research within the subject of ethanol production has focused on two general strains of inquiry. The first includes the refinement of existing ethanol production such as with corn and sugarcane, where researchers in private companies and US government agencies like the EPA and USDA have sought to improve the energy yield while reducing environmental pollution throughout the ethanol life cycle. The second focuses upon novel methods of ethanol production, including non-genetically modified algae, and the process of cellulosic ethanol production. While ethanol production has continually grown since World War II, significant research and investment into new ethanol production strategies blossomed in the early 2000s, where world ethanol production tripled between 2000 and 2007.

Conventional ethanol research is motivated by a mixture of economic and social drivers. Socially, the rising food versus fuel debate (discussed further in the Implications section) has raised questions about the impact of ethanol fuel production on global food prices, where organizations such as the World Bank have asserted that the rising land use of foodstuffs for ethanol production directly contributed to rising global food costs that have significant economic impacts in sub-Saharan Africa (US EPA 2007). Ethanol research is also driven by economic factors, where government agencies and private companies in the United States continue to seek an alternative to corn ethanol, which has a relatively low energy balance score of 2.3. The net energy balance approximates the amount of energy produced given the amount of energy consumed. The net energy balance for each ethanol source will be comparatively assessed later in this chapter. The rapid growth of worldwide ethanol production coupled with these social and economic factors has driven the field's conventional research in order to find an alternative that has a minimal impact on global foodstuffs while improving energy balance ratios and reducing reliance upon fossil fuels.

Experimentation with cellulosic ethanol has occurred since the first cellulosic ethanol plant opening in South Carolina in 1910. However, high production costs have hindered consistent and widespread commercialization (Wang 2009). Using a mixture of wood, grasses, or other inedible plant pieces, cellulosic ethanol is produced via biochemical or thermochemical processing (Pimentel and Patzek 2005). A general production cycle is illustrated in Fig. 2.

Cellulolysis is the process which makes use of lignocellulosic material (or the inedible and structural parts of plants) to create ethanol. Specifically, hydrolysis is used to cleave chemical bonds of the lignocellulosic material using water, where the resulting sugars are eventually fermented and distilled into ethanol (Fujita et al. 2002). The process of cellulolysis is generally subdivided into five stages, including pretreatment, hydrolysis and sugar separation, fermentation, distillation, and dehydration (Lynd et al. 1991; Zhu et al. 2009). The pretreatment phase of cellulolysis is used in order to refashion the biomaterial prior to hydrolysis. Specifically, the lignocellulose within the available biomaterial is treated with chemicals to break its rigid structure, where the chemical method used differs based upon the biomaterial chosen for ethanol conversion. Next, the treated lignocellulosic material is converted via hydrolysis in order to break down the material's sugar molecules in order to isolate those sugar molecules for further fermentation. This generally occurs using one of two forms of hydrolysis, including an acidic chemical reaction or an enzymatic reaction. Chemical hydrolysis has been around since the nineteenth century and involves introducing an acid to the cellulose to separate its sugar molecules.

Fig. 2 Cellulosic ethanol production process. (Image source: US DOE (2007))

The enzymatic process uses enzymes to break down cellulose sugar chains to allow for collection of cellulose sugars.

After hydrolysis, the sugars acquired from hydrolysis are fermented through the use of yeast. These sugars (glucose, sucrose, and fructose) are converted into energy that will be eventually converted into ethanol. After fermentation, distillation of the converted sugars is used to produce 95% alcohol, which allows for eventual conversion into ethanol to be combined with gasoline. Distillation is carried out similarly as with general corn or sugarcane ethanol production. Lastly, dehydration converts the 95% alcohol into an alcohol liquid with a 99.5% ethanol concentration, which makes the ethanol ready for public consumption as vehicle fuel or other gasoline-driven purposes.

The second method of producing cellulosic ethanol includes gasification, or the chemical approach toward producing ethanol from cellulosic material. Rather than using chemical decomposition via cellulolysis, carbon in the cellulosic material is converted into gas, which fuels combustion, and then fermented. This generally occurs in three steps. In the first stage, carbon molecules are broken apart to make carbon monoxide, carbon dioxide, and hydrogen. These molecules are eventually used in fermentation to be converted into energy. Unlike the yeast used for fermentation in the cellulosic approach noted above, gasification uses the *Clostridium ljungdahlii* for fermentation. The bacteria consume carbon dioxide, carbon monoxide, and hydrogen and produce an output of ethanol and water. Lastly, the ethanol and water mixture produced from the *Clostridium ljungdahlii* bacteria are separated via distillation, leaving only the ethanol for commercial consumption.

Cellulosic ethanol is estimated to have an energy balance ranging from 2 to 36, where the large range in energy balance scores reflects different types of biofuels and energy conversion processes used to generate the ethanol (Schmer et al. 2008). This indicates that the method has the potential to produce significantly more energy than corn ethanol (which has an energy balance ratio of approximately 1.3) and potentially even higher than sugarcane (which has an energy balance ratio of 7–8). The technology takes advantage of abundant raw materials, where over 300 million tons of cellulose-containing materials that could create cellulosic ethanol is thrown away each year in the United States. However, the technology remains economically unviable, where cellulosic ethanol has lower energy content than traditional fossil fuels and would cost an estimated $120 per barrel of oil. Research on this technology continues to attempt improvements in energy efficiency and reduction in cost, along with further diversification of feedstock to be used in cellulolysis. For example, kudzu has been suggested as a potential source of cellulosic biomass.

Other than cellulosic ethanol production, an additional alternative method for ethanol production currently under research includes the use of algae. First discussed as a potential fuel source in 1942, German scientists Harder and von Witsch argued that microalgae could be cultured and grown in a controlled setting as a source of lipids for fuel or even food (Harder and von Witsch 1942). In the immediate aftermath of World War II, research regarding the conversion of algae into biodiesel fuel further spread to the United States, Israel, Japan, and England, where motivation for an alternative fuel source remains strong due to fuel limitations throughout the 1940s (Burlew 1953). However, the declining cost of fossil fuels reduced the need for an alternative energy source, although algae fermentation continued to be researched for applications of food and wastewater treatment (Borowitzka 2013).

The international oil embargo in the late 1970s rekindled interest in the development of algal biofuel (DOE). This interest was particularly strong in the United States, which invested $25 million into the Aquatic Species Program over an 18-year period with the intent of promoting a commercialized algal biofuel. However, scientists within this program came to find that natural algae (or those algal organisms lacking any genetic modification via synthetic biology) had several limitations that could hinder large-scale commercialized production, particularly limitations of economically feasible growth in a controlled environment (Sheehan et al. 1998). The final report issued by the Aquatic Species Program suggested that genetic engineering was necessary in order to overcome these natural hindrances and limitations, where a genetically engineered algae would grow and populate faster in a variety of environmental conditions (Sheehan et al. 1998). The Aquatic Species Program was disbanded in 1996, and it was not until a sharp increase in oil prices in the 2000s that funding for such biofuels increased, particularly in the United States, Australia, and the European Union (Pienkos and Darzins 2009). Along with providing domestic energy security, the Australian government has stated that biodiesel production from algal lipids may provide economic opportunities and jobs to various underserved or rural areas (SARDI Aquatic Sciences). By March 2013, the American energy company Sapphire Energy initiated the first commercialized sale of algal biofuels (SARDI Aquatic Sciences).

Today, algae can be used to generate a variety of fuels, where the lipid portion of the algae is converted into biodiesel with the potential for future conversion to ethanol (Ellis et al. 2012). Algae are cultivated and harvested in 1–10 days and can be grown in areas that are unsuited for agricultural production or exposed to untreated wastewater (Chisti 2007). Currently, most research and production of algal biofuel takes place in photobioreactors (a series of glass tubes which are exposed to water) or open ponds, where ponds are less costly than photobioreactors but more vulnerable to contamination (Mata et al. 2010).

Current State of Fuel Ethanol

Ranging from the conversion of corn to fuel in the United States to sugar to fuel in Brazil, the current state of ethanol research and development is driven in an attempt to foster a sustainable fuel source that reduces or eliminates domestic reliance on nonrenewable fossil fuels. A number of different types of feedstock products may be used to generate ethanol, including barley, hemp, sweet potatoes, and cellulose. Yet, production is dominated by corn in the United States and sugarcane in Brazil, with smaller production levels in Europe, China, and elsewhere (Table 1) (Renewable Fuels Association 2016c). Overall, the United States and Brazil accounted for approximately 83% of global ethanol production in 2015.

Global ethanol production has increased on a yearly basis since 2005, and the United States has seen the greatest production rate increase (British Petroleum 2016). Between 2005 and 2015, total ethanol production in the United States increased from 3.9 to 14.9 billion gallons (Renewable Fuels Association 2016c). The majority of US ethanol production occurred in the Mid-West region, where corn optimally grows. Additionally, South America and Central America nearly doubled ethanol production between 2005 and 2015 (British Petroleum 2016).

Table 1 Global ethanol fuel production in millions of gallons produced in 2015 based on Renewable Fuels Association data

2015 World ethanol fuel production (billion gallons)						
Producer	United States	Brazil	Europe	China	Canada	Rest of world
Gallons	14.8	7.1	1.4	0.8	0.4	1.1
Percentage of global production	56%	27%	5%	3%	2%	7%

Energy Efficiency of Conventional and Advanced Ethanol Feedstock

Throughout the ethanol fuel life cycle, one of the fundamental concepts governing the efficiency and viability of turning a specific feedstock into ethanol is energy balance. Specifically, this includes the total amount of energy input into the process of converting biomass against the energy released by burning the ethanol, represented as (Shapouri et al. 2002):

$$\textit{Net}\ \text{energy balance} = \frac{\text{energy produced}}{\text{energy consumed.}}$$

The numerator contains the potential energy that may be used upon burning the created ethanol, while the denominator contains all of the energy invested into producing the ethanol (including field preparation and crop cultivation). An "energy-positive" ethanol is one where the energy produced is greater than energy consumed, while an "energy-negative" ethanol is one where the energy production is lower than the energy consumed. With regard to investments, all energy expenditures in the growth, collecting, drying, and fermentation of biomass are included in the energy balance computation (Agler et al. 2008; Hill et al. 2006; Murphy and Power 2008). Generally, fossil fuel energy is utilized on the investment side of the energy balance equation, where coal, oil, or natural gas is used to convert biomaterial into ethanol (Hill et al. 2006; Murphy and Power 2008). Energy-negative ethanol production methods cost more energy via fossil fuels to create 1 liter of ethanol than would be produced, while energy-positive ethanol production methods offer a net energy gain by the end of the production process. Overall, energy balance is a critical element in determining the efficacy of ethanol fuel production, where if a particular method or feedstock generates a net negative energy balance, it would unlikely be commercialized for the long term. For any potential algal feedstock, the product would eventually have to foster not only a net positive energy balance score but may also need to offer similar or improved energy balance scores to conventional biomaterials should the risks associated with algal ethanol outweigh the risks associated with conventional biomaterials.

Isaias Macedo (1998) conducted studies regarding the energy balance values of corn and sugarcane ethanol, respectively, indicate that sugarcane ethanol has a net positive energy balance number yet corn ethanol is not substantially positive and may even be negative in certain conditions of crop spoilage or improper conversion to ethanol fuel (De Oliviera et al. 2005; Macedo 1998). For corn ethanol, 1 unit of fossil-fuel energy is required to create 1.3 energy units of ethanol (Macedo 1998). This figure was calculated by Macedo in his review of corn ethanol, but more recent analyses suggest that the corn ethanol production processes are becoming more efficient and reach net energy balances ranging from 2.6 to 2.8 (Renewable Fuels Association 2016a, b). While corn ethanol does contribute to a net positive energy balance, the energy improvement is quite limited and may not warrant the environmental degradation caused by harvesting corn and the pollution accrued by converting corn feedstock into ethanol fuel. Sugarcane ethanol is substantially more efficacious, where 1 unit of fossil fuel energy is required to create approximately 8 to 9 energy units of ethanol (Bourne and Clark 2007). This net energy balance indicates that sugarcane is significantly more energy efficient than corn, as it requires significantly less feedstock to produce a greater amount of liquid ethanol to be mixed with various gasoline blends. However, sugarcane may only be grown productively in tropical climates, whereas corn is more flexibly grown across a wider range of climates.

Advanced ethanol production processes may offer higher net energy balances than conventional approaches. By converting cellulosic biomass into ethanol, a wide range of net energy balance values have been presented across the literature. Reported net energy balances of cellulose range from 1.42 to 36, with 36 being a massive net energy producer. The range of values derives from the variance in perennial herbaceous plants that can be harvested for ethanol (Schmer et al. 2008). Naturally occurring cyanobacteria and microalgae that are grown agriculturally can yield net energy balances ranging from 0.7 to 7.8. The range of values here reflects differences in growth environments, where algae with higher net energy outputs may be grown in more suitable environments (Shen and Luo 2011; Brentner et al. 2011) (Table 2).

Overall Observations of Conventional and Newer Ethanol Production Processes

Ethanol fuel additives offer a mechanism to offset petroleum consumption through a variety of feedstock alternatives and production processes. Conventional feedstocks, including corn and sugarcane, have experienced widespread commercialization in the United States and Brazil. More advanced processes, such as those using cellulose and algae, may be more energy efficient but have experienced less commercialization, largely due to their high principal and R&D costs.

Table 2 Net energy balance values for each ethanol source. Higher values indicate greater energy efficiency. The ranges of values reported here reflect the approximate minimum and maximum net energy balance scores presented across prior research and agency reports

Ethanol source	Net energy balance	Source
Corn	1.3–4	Macedo (1998); Renewable Fuels Association, (2016a, b)
Sugarcane	8–9	Bourne and Clark (2007)
Cellulose	1.42–36	Shahrukh et al. (2016); McLaughlin et al. (2011); Schmer et al. (2008)
Cyanobacteria/microalgae (natural)	0.7–7.8	Shen and Luo (2011); Brentner et al. (2011)

A Synthetic Biology Solution to Biofuel Production

Synthetic biology serves as a possible mechanism for improving upon current conventional and advanced ethanol feedstock options. The use of synthetic biology to improve ethanol production is similar to existing conventional research in that its motivation is to find an economically feasible feedstock source that is energy efficient. While synthetic biology applications mirror conventional research in the cultivation of algae as a biomass for fuel production, the technology differs in that algal blooms are specifically engineered to enhance fermentation and photosynthesis processes, increase lipid content, increase pathogen resistance, produce higher-value co-products, and/or diminish unwanted cellular regulation (Georgianna and Mayfield 2012; Gimpel et al. 2013). Overall, the primary goal of synthetic biology's algal ethanol option is to dramatically reduce the energy needed to convert biomass into fuel ethanol such that engineered algae could produce significant amounts of algal oil (an immediate precursor to ethanol fuel) without the significant fossil fuel and manpower resources needed to produce ethanol from conventional biomass. With these R&D aims, algal ethanol is anticipated to be more energy efficient than corn or sugarcane, where engineered algae would only require initial start-up energy costs to produce several substantial harvests of various ethanol fuels. Synthetic biology ethanol technologies aim to improve the existing limitations of corn ethanol (i.e., increasing the net energy balance), sugarcane ethanol (i.e., desensitize feedstock to grow in diverse environments), cellulosic ethanol (i.e., reduce downstream production costs), and naturally occurring algal ethanol (i.e., increase net energy balance).

The synthetically engineered algal ethanol process would be accomplished by converting the algae's lipids into biodiesel, which is identical to the process noted above for non-engineered algal oils. Subsequently, the algal cells' carbohydrates can be fermented into bioethanol in a process very similar to existing conventional practices in corn or sugarcane.

Synthetic biology was proposed by Craig Venter in 2011 as a tool to make algal cells more economically viable and technologically feasible as an ethanol production source while improving the capabilities of algal ethanol production in terms of energy requirements, environmental impact, and economic potential. By fine-tuning the genome of specific algae using synthetic biology techniques, it is possible to create a modified species of algae that is a highly cost-effective alternative to other forms of biomass while being compatible with existing bioethanol manufacture and supply infrastructures. For example, where many existing bioethanol products have low energy density and are incompatible with existing fuel infrastructure (Stephanopoulos 2007; Atsumi et al. 2008), Craig Venter of Synthetic Genomics Inc. claims that engineered algae could be engineered and developed to produce 5–10 times more fuel per acre than contemporary feedstock. Likewise, where biodiesel is plagued by issues such as high cost and limited availability of necessary biomass, engineered algae are sustainable in that algae can be manipulated to continually produce ethanol via sunlight without killing the algae cell in general

(Demirbaş 2002). In 2009, ExxonMobil funded and began a collaborative effort with Synthetic Genomics Inc. In 2017, the pair announced a breakthrough in advanced biofuel production—they increased algae's oil content from 20% to 40% (Ajjawi et al. 2017).

Genetically manufactured algae can serve as a renewable, economically viable, and energy-efficient method of replacing limited fossil fuels (Georgianna and Mayfield 2012). Additionally, the ability to engineer such algae to have similar properties to petroleum-based fuels allows for its use in existing transportation infrastructure, which can limit indirect costs involved in switching fuel sources (Peralta-Yahya et al. 2012). Such algae would be required to exhibit certain characteristics, including (Alper and Stephanopoulos 2009):

(a) High substrate utilization and processing capacities
(b) Fast pathways for sugar transport
(c) Good tolerance to inhibitors
(d) High metabolic fluxes, and
(e) Producing a single fermentation product

As such, while the potential for algae to serve as the next wave of ethanol biofuels is apparent, it is still uncertain how much biosynthesis and genetic manipulation is required to produce an "ideal" product. Additionally, tens of thousands of algae species exist, further complicating the identification of an ideal candidate for further research and use.

Proposed Role of Synthetic Biology in State of Fuel Ethanol Production

Synthetic biology has emerged as a technical approach to enhance algal ethanol production, aiming to make algal ethanol more energy efficient, regenerative, and less costly. However, because of the novelty and uncertainty surrounding synthetic biology, it is unclear whether synthetically engineered algae are a viable bioethanol alternative. To determine optimal ethanol feedstock sources and processes, it is necessary to comparatively review the alternatives using risk assessment. The risk assessment should include traditional quantitative assessments but further be informed by a "solution-focused" orientation to risk. By using SFRA, the scope of assessment expands beyond the cost considerations emphasized in the EPA's latest Renewable Fuels Standards Program, and the unique benefits of each feedstock alternative are compared. Existing assessment protocols are not fully able to capture the complexity of ethanol production processes, and traditional risk assessments also have difficulty dealing with the uncertainty of synthetically engineered organisms (Trump et al. 2019). A solution-focused risk assessment provides a lens to think comparatively and holistically about the impact of synthetically engineered biofuels. As the problem is complex in nature, assessments need to be comprehensive and consider a variety of factors. The intent of this assessment is not to provide a conclusion on the viability or ethics of synthetically engineered algal ethanol, but rather to pave the way for thinking about risks as the technology continues to develop.

Method: Solution-Focused Risk Assessment

Thinking about complex operations and risks involved in the energy sector is difficult due to its multi-faceted implications for the economy, the environment, and human health. Because data on emerging technologies are limited due to their novelty, it is challenging to derive accurate estimates of the environmental and social impacts of emerging energy technologies. However, uncertainty analysis can help evaluate the hazard and exposure scenarios associated with emerging energy technologies, in this case synthetic biology-enabled products. As a first step in exploring the potential risks imposed by emerging algal ethanol production, relative and comparative assessment characterizes the potential benefits and unique risks posed by engineered algae relative to the risks of conventional sources. SFRA is one platform to review various implications that a synthetic biology option for biofuels might have, including comparative consideration of technological risks, costs, and benefits. These implications can be compared across ethanol feedstock options to determine which option optimally satisfies the goal of attaining a cheap, renewable, efficient energy source with minimal downside risks to human health and the environment. A synthesis of qualitative and quantitative information will be included to compare conventional and synthetic biology options for fuel ethanol. The information will be divided into four factors:

1. Sustainability
2. Economic implications
3. Environmental implications
4. Novel risk potential of synthetic biology

Traditional risk assessment quantifies the safety of a product or process according to hazard, exposure, and effect data (EPA 2017). This risk assessment approach helps identify scenarios in which products or processes are generally considered safe enough for commercial use. However, traditional quantitative risk assessment does not fully consider and weigh the costs and benefits of technological alternatives, especially those that are just emerging. While the method can deem whether a current technology is safe or unsafe, it bypasses the opportunity to solve problems by considering unique benefits that may result from an emerging technology—particularly as data availability on a newer approach to an old problem may be limited. SFRA utilizes concepts of traditional quantitative risk assessments and considers whether there are potential emerging technologies that can be developed and commercialized for a more optimal outcome. Thus, SFRA includes both existing quantitative data and potential qualitative information in order to compare technological alternatives.

In this chapter, the development and use of synthetically engineered algal ethanol is considered through the SFRA approach. The possible benefits and impacts of using synthetic biology to enhance algal ethanol are compared against conventional and advanced ethanol production processes. A solution-focused assessment has been applied to multiple factors that will determine the viability and efficacy of ethanol production alternatives. The analysis presented in this chapter primarily serves as an initial framework for which to compare ethanol production sources; future sensitivity and uncertainty analyses should be conducted to refine the risk and benefit parameters presented here.

A literature search for relevant data was conducted using peer-reviewed articles and government agency and private sector reports to inform each assessment. The assessment constructs and measures each ethanol feedstock alternative by the four factors, defined as:

1. *Sustainability:* determined by land use and resource availability by geography and climate
2. *Environmental implications:* determined by greenhouse gas emissions and water requirements
3. *Economic implications*: determined by cost per gallon produced and direct and indirect employment rates
4. *Novel risk potential of synthetic biology:* includes qualitative information related to environmental, legal, and technological risks unique to synthetically engineered algae

Results of Solution-Focused Risk Assessment

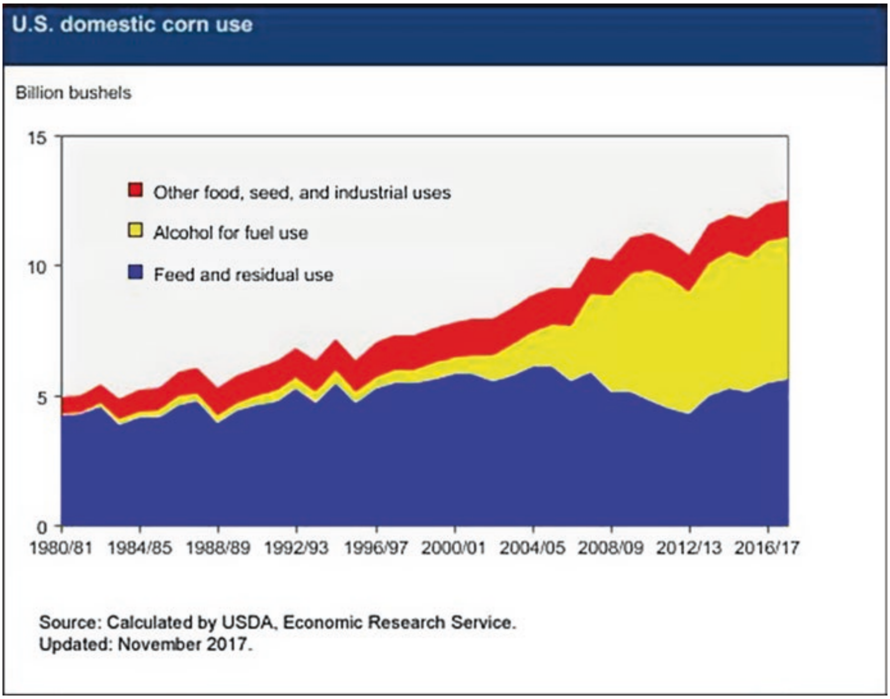
Based on data compiled through the literature review, conventional feedstock options and synthetically engineered algae were holistically compared.

Sustainability Metrics and Data

To determine the sustainability of ethanol feedstock, the available supply of ethanol resources and land use requirements were assessed. Available supply was determined by where ethanol feedstock resources are geographically located and to what magnitude. Land use requirements were assessed by the volume of ethanol produced for each feedstock alternative according to liters (L) produced per hectare (ha) per year (yr). This is a common metric used across the literature to assess land use; any resources that reported land use data with different units were converted to liters per hectare per year. High land use requirements contribute to the ecological footprint of an energy source, resulting in the potential loss of biodiversity and increased erosion (Dias de Oliveria et al. 2005).

Renewable fuel sources are defined by the US Department of Energy as "…combustible liquids derived from grain starch, oil seed, animal fats, or other biomass; or produced from a biogas source, including any non-fossilized, decaying, organic matter capable of powering spark ignition machinery" (Alternative Fuels Data Center 2017). These fuel sources are regenerative, unlike depleting sources of coal and petroleum. Sustainability is necessary to consider for emerging energy technologies, as fossil fuels will eventually be scarce and humans will increasingly need to utilize alternative fuel sources. Bioethanol is one possible option.

In the United States, corn feedstock drives the majority of biofuel production. In 2015, corn feedstock led to the production of 14,659 billion gallons of biofuel—a vast majority relative to the 450 billion gallons produced from wheat feedstock and the 1.5 billion gallons produced from sugarcane (Bergtold et al. 2016). However, this production does not come without costs. In the United States in 2016, almost ten million acres of land was used to grow corn. One-third of US domestic corn is used for alcohol for fuel use (Fig. 3), suggesting that over three million acres of US land is used for corn ethanol production (USDA Economic Research Service 2017). Thus, corn ethanol is a land-intensive production process that utilizes land that may



Source: Calculated by USDA, Economic Research Service.
Updated: November 2017.

Fig. 3 US domestic corn use estimates that nearly one-third of domestic corn is converted to alcohol for fuel use otherwise be used for food production. Sugarcane ethanol is primarily produced in Brazil; in 2015, Brazil's sugarcane ethanol industry contributed to 28% of global ethanol production (Renewable Fuels Association 2016a, b). Despite Brazil's high sugarcane ethanol output, only 4.6 million hectares of Brazil's total 851 million hectares of land area is utilized for ethanol production. Thus, only 0.5% of Brazilian land area is needed for ethanol production (UNICA 2016). Additionally, Brazil has undertaken agro-ecological zoning regulations that ensure sugarcane expansion is sensitive to biodiversity and native vegetation (UNICA 2016). Relative to conventional production's land use, it is estimated that only 4% of US land would be needed for algae to replace the energy supply of *all* domestic and imported petroleum used in the United States (Georgianna and Mayfield 2012). The potential benefits of algal ethanol production are further pronounced considering that even if all US corn and soybean (another conventional ethanol feedstock) were dedicated to biofuel production, this would only meet 12% of US gasoline demand and 5% of diesel demand (Hill et al. 2006).

To directly measure land use requirements for each feedstock alternative, a measure of liters of ethanol produced per hectare in a year was included in this comparative review (Table 3). Higher values indicate that greater volumes of ethanol can be produced from a hectare-sized area of land. Corn has the lowest volume output of ethanol per hectare per year at 4600 L produced (Georgianna and Mayfield 2012). Sugarcane has the second lowest output, at 9000 L per hectare (Goldemberg 2008). The advanced production feedstock yields significantly greater ethanol output than conventional feedstock, and synthetically engineered algal ethanol is estimated to produce the highest volume of ethanol per hectare in a year at 93000 to 112,000 liters produced (Waltz 2009).

Environmental Impact Metrics and Data

In addition to sustainability considerations, the environmental impacts of corn, sugarcane, cellulose, and algal ethanol were assessed using two factors: greenhouse gas emission rates and water requirements for production. These metrics were included as they are commonly used in environmental life cycle assessments (Georgianna

Table 3 Liters produced of ethanol per hectare per year for each ethanol feedstock alternative

Ethanol source	Land use (liters per hectare)	Source
Corn	4600	Georgianna and Mayfield (2012)
Sugarcane	9000	Goldemberg (2008)
Cellulosic biomass	1000–2000	Robertson et al. (2017); Sanford et al. (2017)
Microalgae (natural)	36,000–115,000	Georgianna and Mayfield (2012); US Bioenergy Technologies Office (2016)
Cyanobacteria/microalgae (synthetic)	93,000–112,000	Waltz (2009)

and Mayfield 2012). Greenhouse gas (GHG) emissions are necessary to consider because their release into the atmosphere adversely traps heat and subsequently increases the global average temperature. Between 2005 and 2015, ethanol production in the United States increased from 3.9 to 14.9 billion gallons (Renewable Fuels Association 2016c). Given the increasing ethanol production rate, it is critical to asses which ethanol production processes optimally reduce carbon dioxide and other GHG emissions. Additionally, the fuel ethanol industry could have significant impacts on global GHG emissions, as transportation contributes to approximately 29% of total GHG emissions in the United States and 14% of total emissions worldwide (EPA 2018). Greenhouse gas emissions are a critical consideration, as the rate of global GHG emissions increased by approximately 2.7% from 2017 to 2018, reaching 37 billion tons (Global Carbon Project 2018).

In this analysis, GHG emissions for each ethanol source are presented as a rate change relative to petroleum GHG emissions. For each source, a variety of percent changes were presented across the literature. Therefore, when applicable, this data is presented as a range from the lowest reported percent reduction in GHG emissions to the highest reported reduction in GHG emissions.

Table 4 represents the percent reduction rate of GHG emissions relative to petroleum for each ethanol feedstock source. All conventional and advanced feedstock options reduce GHG emissions relative to petroleum. Conventional feedstock options are reported to reduce GHG emissions by a lesser percent than advanced options; specifically, some corn ethanol estimates yield a 21.8% reduction rate, whereas estimates of cellulosic biomass reach 89–94% reduction rates. Microalgae reduce GHGs by about 70%, thereby making them on par or less emission-intensive than other ethanol sources. Data on synthetically engineered algal ethanol is still in development, but assuming synthetic biologists fulfill their aims of engineering more efficient algae that consume CO2 as a primary food source, synthetic algal ethanol production has the potential to serve as a greenhouse gas mitigation technique.

Additionally, water requirements are assessed for comparison. Water requirements are presented in terms of gallons of water required for each gallon of ethanol produced. The resulting values reflect aggregated water input required for each stage of the production cycle, including harvesting, hydrolysis/liquefaction, fermentation, distillation, and transportation. Like the reported GHG emission rates, a range of gal water/gal ethanol is presented for each feedstock alternative.

Table 4 Percent reduction in greenhouse gas emissions relative to the emission rate of petroleum

Ethanol source	Percent reduction in GHG emissions (relative to petroleum)	Source
Corn	22–76%	Renewable Fuels Association (2016c); EPA (2007)
Sugarcane	56–80%	EPA (2007); Junqueira (2017)
Cellulosic biomass	89 –94%	Schmer et al. (2008); Wang et al. (2011)
Microalgae	69%	Algenol (2017)

On average, 3–15 gallons of water is required to produce 1 gallon of ethanol (Wu et al. 2009). The estimated number of gallons required to production 1 gallon of ethanol for each feedstock option is shown in Table 5. The ranges are quite spread for many of the feedstock options, largely because climatic and environmental conditions influence the amount of water needed for feedstock harvesting and cultivation. For instance, under optimal environmental conditions, corn growth would require less water than it would under suboptimal environmental conditions (i.e., high temperatures). Based on data found through the literature review, conventional ethanol production has higher water requirements than advanced ethanol production methods. Naturally occurring microalgae potentially have the lowest water requirements, as only 0.6 gallons of water are needed to produce a gallon of ethanol (Martín and Grossmann 2013). However, an upper bound suggests that microalgae could require up to 964 gallons of water to produce 1 gallon of ethanol. Like conventional feedstock, the estimate is dependent on growth conditions and specific production processes. Should the goals for synthetically engineered algae be achieved, the water requirements of microalgae could be further reduced, particularly through the closed-feedback growth cycles of photobioreactors. Additionally, algae can be engineered to use and recycle non-potable water, such as saltwater and brackish water. While conventional feedstock requires freshwater for cultivation, algae reduce dependence on freshwater consumption. In the future, the range of water use for each ethanol source should be further analyzed in terms of probability distributions over the range, and Monte Carlo simulations could be used to derive the average and the reasonable ranges of performance for each ethanol source.

Costs and Social Well-Being Implication Metrics

While sustainability and environmental impact assessments are critical to include in the SFRA, socioeconomic implications were assessed to develop the holistic approach to the feedstock comparison. Costs of each feedstock were determined by the cost per gallon of ethanol produced, and social implications focused on job creation and loss. Specifically, costs per gallon produced are calculated using a metric called the gasoline gallon equivalent. The energy density of ethanol is about 60–66%

Table 5 Water requirements for feedstock type based on gallons of water needed to produce 1 gallon of ethanol

Ethanol source	Water use (gal water/gal ethanol)	Source
Corn	1–324 gal	Wu et al. (2009); National Academy of Sciences and National Research Council (2012)
Sugarcane	927–1391 gal	Wu et al. (2009)
Cellulosic biomass (switchgrass)	1.9–9.8 gal	National Academy of Sciences and National Research Council (2012)
Microalgae (natural)	0.6–964 gal	Martín and Grossmann (2013)

that of gasoline, as gasoline yields approximately 34 MJ/L and ethanol yields approximately 18–23 MJ/L (Jolly 2001). Thus, researchers often use the gasoline gallon equivalent to compare the cost of different energy resources, which controls for energy output by volume (EIA 2017a, b). The gasoline gallon equivalent determines the cost per gallon by including feedstock cost, equipment costs, and final product yields. Therefore, it accounts for facility and equipment costs that may impose capital cost restraints for a feedstock option. All prices were adjusted to the 2016 US dollar. It is important to note that these cost estimates do not include any potential subsidies or government-imposed financial incentives.

Table 6 presents the cost per gasoline gallon equivalent for each feedstock type. These prices capture current production costs given energy density relative to gasoline. These costs are not, however, would not necessarily be consumer-facing, as they do not account for regulation or subsidies. The average cost of a gallon of gasoline in 2016 was $2.43 (EIA 2016). The cost per gasoline gallon equivalent of ethanol in 2016, averaging across all ethanol feedstock options, was estimated to be between approximately $2 and $2.50 in the United States (EIA 2017a, b; AFDC 2017). Conventional ethanol feedstock cost estimates are lower than advanced ethanol feedstock, with corn's GGE cost estimated to be less than gasoline itself (USDA 2006). Cellulosic biomass feedstock stands as the least costly advanced ethanol production process, at $2.20 to $5.50 GGE. Microalgae, whether through hydrothermal liquefaction production processes or the current industrial "state-of-the-art" technology, are the most expensive ethanol feedstock. As these cost estimations are accounting for facility and equipment costs, these high costs are likely driven by research and development equipment investments. The industrial state-of-the-art synthetic algal ethanol currently costs about $13–17 to produce per gallon, which is significantly more expensive than conventional feedstock options. This expense may drive consumers away, as they would purchase cheaper ethanol derived from different feedstocks; however, should the other risks (e.g., land use) of algal ethanol outweigh those imposed by conventional feedstock options, government subsidies on algal ethanol could be imposed. Additionally, it has been suggested that algae

Table 6 The cost per gasoline gallon equivalent for each ethanol feedstock type

Energy source	Cost per gasoline gallon equivalent (GGE)	Source
Gasoline	$2.43	EIA (2016)
Ethanol (general)	$1.96–$2.53	EIA, (2017a, b), AFDC, Clean Cities Price Report (2017)
Corn	$1.21–$1.23	USDA (2006)
Sugarcane	$0.95–$2.76	USDA (2006)
Cellulosic biomass	$2.20–$5.49	U.S. DOE (2015); Adusumilli et al. (2013)
Microalgae (hydrothermal liquefaction process)	$2.11–$7.23	Zhu et al. (2013)
Microalgae (industrial state of the art)	$13.35–$17.00	U.S. DOE (2017)

would not be killed during the collection of ethanol, allowing for a continual use of the organism to produce fuel (Georgianna and Mayfield 2012). While it is perhaps too early to be certain, it is likely this ability to continuously reutilize engineered algal cells would contribute to a further decline in cost per gallon yield, as the same cells would produce several harvests of ethanol fuel with only site maintenance and the provision of algal food to keep production going.

In addition to this production cost comparison, economic considerations such as direct and indirect employment rates are valuable assessments to gauge how conventional and advanced ethanol production markets might respond to a disruptive emerging ethanol production technology. Conventional feedstock production processes actively employ thousands of individuals in the United States and over a million in Brazil. Therefore, should an emerging technology, such as synthetically engineered algal ethanol, erupt, these markets could be significantly disrupted. Ethanol production in 2015 led to the employment of nearly 86,000 direct jobs across the United States and added $44 billion to the US gross domestic product (GDP) and $24 billion in household income (Renewable Fuels Association 2016a, b). In addition to direct employment and profit, corn ethanol market further entails indirect economic impacts and employment opportunities. When direct, indirect, induced, construction, agriculture, and R&D jobs supported by ethanol production are included, the number of employment opportunities in the United States was estimated at more than 357,400 jobs in in 2015 (Urbanchuk 2017). It is important to note that this estimate may not capture all the jobs that were already displaced in the shift from corn for food to corn for ethanol.

Brazil produces the majority of global sugarcane ethanol and remains the second leading producer of ethanol worldwide (Renewable Fuels Association 2016a, b). The Brazilian sugarcane industry is comprised of three main sectors: sugarcane cultivation, sugar production, and ethanol production (Moraes et al. 2015). The sugarcane industry as a whole employed approximately 1.2 million workers in 2015 and generated $36 billion USD in gross annual revenue (UNICA 2016). In Brazil, the number of new and closed sugarcane ethanol mills has been steadily decreasing, likely reducing the number of employees hired by the industry. For instance, 430 mills were running in 2010, while only 383 mills existed by 2016. For each year between 2005 and 2011, there were more net sugarcane ethanol mill openings than closures; however, since 2012, there have been more net mill closures than openings (Renewable Fuels Association 2016c). The industry's declination in active sugarcane production mills may be attributed to changes in the Brazilian political and socioeconomic climate (Granco et al. 2015).

Should synthetically engineered algae ethanol technology become more widespread and commercialized, it could swing the fuel economy in both beneficial and disruptive ways. As a net positive, the introduction of synthetic algal biofuels into several nations which currently do not produce significant corn, sugar, or cellulosic ethanol would enable such states to produce their own domestic renewable fuel. This would be particularly advantageous to those states with limited arable land or few crops with a significant positive energy balance score, as algal blooms are able to produce ethanol in a variety of terrains as long as they have access to sunlight, water, and CO_2 (Georgianna and Mayfield 2012; Darzins et al. 2010).

However, countries whose GDP is significantly bolstered by their current ethanol industry may be negatively impacted by the economic disruption of algal biofuel on their existing ethanol production. In Brazil, ethanol production has declined already in part because of the expansion of corn ethanol production in the United States. If synthetically engineered algal ethanol expands in such a robust way that it can be grown on non-arable land, the United States and Brazil may face declining rates of ethanol exportation. Further, the synthetic biology approach will likely limit the number of employees required, as the algae will need less maintenance than corn or sugarcane. Therefore, the number of direct ethanol production jobs will potentially decrease, causing employment rates to drop particularly in the US Midwest and Brazil.

Novel Risk Considerations of Synthetic Biology Approaches

Each of the conventional, advanced, and emerging ethanol feedstock options poses some degree of unique drawbacks. An observed drawback of increased conventional ethanol production includes a corresponding increase in prices of crops used for fuel, which can lead to a rise in food prices locally and globally and diminished food production (Babcock 2012; Inderwildi and King 2009). Additionally, while studies indicate a reduction in CO_2 emissions by corn ethanol in comparison to unleaded gasoline and reductions in CO_2 emissions by sugarcane ethanol, the conversion of fields for crop harvesting contributes to a significant one-time spike in CO_2 that may take decades to balance out with the fuel's reduced CO_2 (Bourne and Clark 2007; Rosenthal 2008).

Synthetically engineered algae may offer distinct benefits over conventional and advanced production processes, such as decreased land and water requirements, increased energy efficiency, and the ability to grow on non-arable land. However, there are unique risks potentially imposed by this emerging technology that are not relevant to conventional ethanol and advanced ethanol feedstock conversion. These novel risks may be present in the production cycle itself or during subsequent interaction with the natural environment. Considerations include how synthetically engineered algae may yield biosecurity and biosafety risks. Biosafety risks largely apply to the concept of horizontal gene transfer, which is defined as the transfer of genes between organisms in a manner other than traditional reproduction. Synthetic biology technologies in particular face this risk as horizontal gene transfer is a common and "somewhat uncontrolled" trait in the microbial biosphere (Cardinale and Arkin 2012). If engineered algae cells transfer synthetic information into the natural world, unanticipated and potentially adverse consequences could result (Cardinale and Arkin 2012; Michalak et al. 2013). Therefore, horizontal gene transfer may instigate risks to the biodiversity of the natural environment that are not yet well characterized. Proper containment of engineered organisms is critical yet difficult as research efforts have focused on mutations at the micro-organismic level. Photobioreactors offer greater containment security than open pond systems but also entail higher capital costs of instalment.

Biosecurity concerns present risks of nefarious agents or bioterrorists harnessing synthetic biology mechanisms and technologies to create biological weapons with devastating consequences (Schmidt et al. 2009; National Research Council 2004). Biosecurity entails concerns of "dual use"—where synthetic biology technologies designed to benefit humans and the environment are deliberately misused for human or environmental harm. As synthetically engineered algae present an opportunity as an energy resource, risks to domestic energy security may be imposed should the technology be misused.

Additionally, as engineered algae are in its research and development phase, it is not yet possible to ensure that researchers will be able to engineer algae in an entirely predictable, consistent, and controlled manner. Off-target gene editing may occur, resulting in synthetically engineered algae that do not yield ethanol as desired. Substantial genetic modifications of cells may impose adverse consequences to humans and the natural environment (Mukunda et al. 2009; Moe-Behrens et al. 2014). To overcome similar research and development challenges associated with genetically modified algae used to produce algal ethanol, Henley et al. (2013) considered a range of impacts that genetically modified algae could have in the natural ecosystem. By listing conceivable risks associated with genetically modified algae as well as non-genetically modified algae, they were able to quantitatively and qualitatively compare natural and modified algae across a variety of hypothetical ecological, economic, and health-related risks. Henley et al. (2013) recommend that risk assessment protocols must first develop open mesocosm experiments for testing, prior to mass cultivation (Seager et al. 2017). Additionally, testing protocols should be adapted to the potential site of mass cultivation of genetically modified algae, which should be marked with detectable genetic markers. We recommend that synthetically engineered algal ethanol risk protocol uses similar testing protocol that is sensitive to local environments and ecosystems.Finally, the synthetic biology ethanol industry faces internal technical risks. Even if the algae are synthetically engineered to provide optimal benefits with minimal associated risks, commercial success is not guaranteed. For engineered algal ethanol to outsource conventional production processes, the technology will need to be massively scaled up. This will require large amounts of time and money for further research and infrastructure development (Connor and Atsumi 2010).

Discussion

Pursuing ethanol as a renewable alternative (or complement) to petroleum has demonstrated environmental benefits, such as reduced greenhouse gas emissions, and can lead countries with limited oil reserves toward oil independence. Synthetic biology offers opportunities to enhance ethanol production in such a way that it bypasses some of the current limitations facing conventional and advanced production processes. Synthetic biologists are engineering algae to achieve a more efficient, renewable fuel source as an alternative to diminishing fossil fuels. Specific to synthetically engineered algae, development and containment uncertainties may lead to biosecurity and biosafety concerns, such as unintended mutations, horizontal gene transfer, and negative human and environmental health consequences. Synthetically engineered algal ethanol also entails high capital costs of investment and may disrupt conventional ethanol production processes that US and Brazilian economies benefit from.

Beyond the hazard, exposure, and effect assessment set forth by traditional risk assessments, a solution-focused risk assessment was used to compare synthetically engineered algal ethanol to conventional and advanced ethanol feedstock options. SFRA methods provide a holistic and comparative assessment as to which ethanol feedstock pursuits offer the greatest benefits and reduced risks. Thus, a solution-focused risk assessment compared each feedstock option across four factors: sustainability, environmental implications, economic implications, and novel risk potential for synthetic biology. The SFRA method builds off traditional risk assessment in that it encompasses both quantitative data and qualitative information related to the safety and net benefits of multiple products or processes that each fulfills the same human need or want.

Based upon an SFRA of engineered algal ethanol against conventional and advanced ethanol production alternatives, there are potenital benefits of continuing research and development on engineering algae to increase the global renewable energy supply. Synthetically engineered algae are demonstrated to be less land intensive than other feedstock options (Table 3), and they allow for more net energy production in a vast array of environments and climates, as the algae harvesting and cultivation take place in controlled laboratories. The controlled growth process makes algae robust and capable of growing on non-arable land, which is beneficial for countries without domestic oil reserves or land capable of growing corn or sugarcane. Additionally, synthetically engineered algal ethanol yields higher volumes of ethanol per land area as opposed to conventional ethanol feedstock options, which are land intensive. Synthetically engineered algal ethanol is also being designed to have a higher net energy balance than other feedstock options, particularly in that algae are being engineered to produce ethanol without dying. Therefore, less energy will be expended into the harvesting and cultivation phases of the production cycle. According to quantitative estimations of GHG emissions and water requirements, synthetically engineered algal ethanol seems to outperform other conventional and advanced ethanol feedstock options. Thus, the associated benefits of engineered algal ethanol exemplify progress toward a sustainable, efficient renewable energy source.

The economic and environmental implications of these bioethanol feedstock options are sensitive to specific geographic locations of production and to the technologies used. To further refine the estimates presented here, mathematical analyses can be used to quantitatively compare bioethanol feedstock options and production technologies. In prior research, probabilistic analyses have been used to simultaneously compare multiple objectives associated with bioethanol production (Kostin 2013; Amigun et al. 2011). Kostin et al. (2012) assessed Argentina's sugarcane ethanol industry by developing a decision support tool for strategic supply chain management, taking into account both economic and environmental parameter constraints and uncertainties. Three mathematical models were used (deterministic, stochastic, multi-objective) for optimal industry planning and design. This sort of quantitative analysis could be applied to other countries and compare a variety of feedstock options, as was performed in this SFRA. An extension of this SFRA that incorporates stochastic models could handle levels of uncertainty in product demand, economic implications, and environmental implications that would better reflect the sensitivities and uncertainties of particular geographies and economies. Similarly, Amigun and Gorgens (2011) conducted a quantitative risk and cost assessment of advanced bioethanol production in South Africa using a stochastic Monte Carlo analysis. Monte Carlo analysis was used to quantify economic risk outcomes across three production technologies under a range of economic parameters. Both the mathematical programming and Monte Carlo approaches to sensitivity and uncertainty analyses of bioethanol feedstocks could include the economic and environmental benefits and risks presented here. For instance, the land use measures presented here are largely dependent on local environments and geography. To assess a similar problem, Tenerelli and Carver (2012) used multi-objective and uncertainty analyses for agro-energy spatial modeling to assess the land capabilities of various perennial crops used for energy. Their model served to assess the potential of different topographies and provided a range of these potentials for energy crop conversions. An uncertainty analysis was performed that simulated the influence of input data and model parameters (Tenerelli and Carver 2012). A similar method and simulation as applied to bioethanol feedstock options would aid in making more accurate risk reduction calculations than the general ranges provided here.

Future research that merges SFRA with quantitative sensitivity analyses will help identify decision thresholds specific to different geographies and economies for which particular feedstock options may have net risk reductions relative to other bioethanol feedstock options and fossil fuels. Prior research on bioethanol feedstock comparisons has shown that the net environmental impact of ethanol fuel depends on the structures of individual production processes, whose predicted outcomes are heavily influenced by the parameterized calculations used (Börjesson 2009). Therefore, to further develop this SFRA, a sensitivity analysis of the four factors (sustainability, environmental implications, economic implications, and novel risk potential for synthetic biology) will help determine optimal place- and economy-specific feedstock options.

While these sensitivity analyses are useful for known risks and benefits, the potential novel risks associated with synthetically engineered algae must also be considered. Emerging technologies bear the brunt of uncertainty and complexity, making it difficult for developers or risk analysts to quantify the risks associated with a technology that has not yet experienced commercialization. Synthetic biology involves various uncertainties regarding the likelihood and magnitude of adverse effects. Despite the potential benefits that synthetic biology products may offer relative to conventional technologies, the novel risks and uncertainties may slow regulation, thereby limiting development and market diffusion (Trump 2017). An adaptive approach to regulation can help governments adjust policies and regulations in an iterative manner as more information is acquired on genetically engineered algae (Greer and Trump 2019).

A more specific approach to quantifying specific risks associated with synthetic biology products is outlined in Trump et al. (2018). This approach could be applied to synthetically engineered algal ethanol and potentially serve to reduce some of the uncertainties and close some of the gaps in knowledge that currently exist. Under this framework, it is first necessary to identify each potential hazard associated with the engineered algal ethanol while understanding that some hazards may be unpredictable. Then, it would be necessary to pair each hazard with its individual risk characterization, which would be independently calculated. For the risk characterizations of each hazard, it is recommended that prior research is used to draw boundaries on plausible values of exposure effects; in this case, parameters might include the proliferation rate of the synthetically derived algae (relative to the proliferation rate of naturally occurring algae). Then, explicit experimental procedures can allow for measuring these parameters where the risk outcome (e.g., loss of containment) is sensitive to the parameter. The engineered algae used to produce ethanol can be tested in a freshwater source, such as a contained water source that is similar to the natural environment. The interaction of the algae with the natural environment will help estimate the magnitude and severity of the unique risks posed by the algae. The environmental risk can be further studied by sensitivity analyses that simulate the engineered algae breaking the contained testing area to potentially more sensitive, natural environments with greater biodiversity.

This approach to considering novel risks associated with algae has been similarly evaluated by Henley et al. (2013) in their consideration of the potential ecological, economic, and health-related implications of genetically modified algae. They focus particularly on the risk of horizontal gene transfer but predict that most traits introduced into genetically modified algae are not likely to hold a comparative advantage to naturally occurring algae, which would result in a low ecological risk. Henley et al. (2013) outline all possible risks associated with genetically modified algae—a very similar approach to that proposed by Trump et al. (2018). Henley et al. (2013) propose that coupling continual monitoring of genetic and mechanical containment strategies with novel cultivation techniques (e.g., matching genetically modified algal traits to unnatural conditions) will help reduce risks. Thus, through monitoring and mesocosm experimentation in contained areas, it is possible to get a sense of how genetically modified and synthetically engineered algae would interact with the natural environment. Despite the potential risks, continued and controlled experimentation is necessary to determine whether the benefits posited by synthetically engineered algae truly outweigh the expected and unknown associated risks.

While studies of this sort continue, especially within private corporations pursing synthetically engineered ethanol production mechanisms, future researchers and developers in this space should carefully consider how to prioritize and categorize hazards. For instance, if the algae had the potential for horizontal gene transfer with humans that would affect human body chemistry, this type of risk should be mitigated before synthetically derived algal ethanol is aggressively pursued. In assessing and managing potential risks such as horizontal gene transfer, a Bayesian approach to uncertain biogeography's and species distribution could be used (Landis et al. 2013). Under this approach, Markov chain Monte Carlo analyses are used over possible biogeographies, which allows the parameters of a biographic model to be estimated and compared (Landis et al. 2013). Specifically, this Bayes approach uses collected data to estimate the joint posterior probability of parameters to develop realistic biogeographic models (Landis et al. 2013). This approach could help estimate the proliferation and propagation of synthetically derived algae from data that have already been collected on the organism.

Based on the present SFRA approach and future integrations with more quantitative risk analyses, synthetically engineered algal ethanol may be a viable renewable energy resource that could offset fossil fuel consumption and make it possible for more countries to establish energy independence. Future research on ethanol production should continue to compare both the risks and benefits of the spectrum of different ethanol feedstock options. Solution-focused risk assessment offers a platform to make this comparison holistic and consider the impact that emerging synthetic biology technologies will have on conventional energy production and on the externalities accompanying it.

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