Using Microbes to make Biofuels

Grandad: When we visited our relatives during the spring vacation, I saw a sticker on the airplane that said 'sustainable biofuel'. What is biofuel and is it good for the environment?

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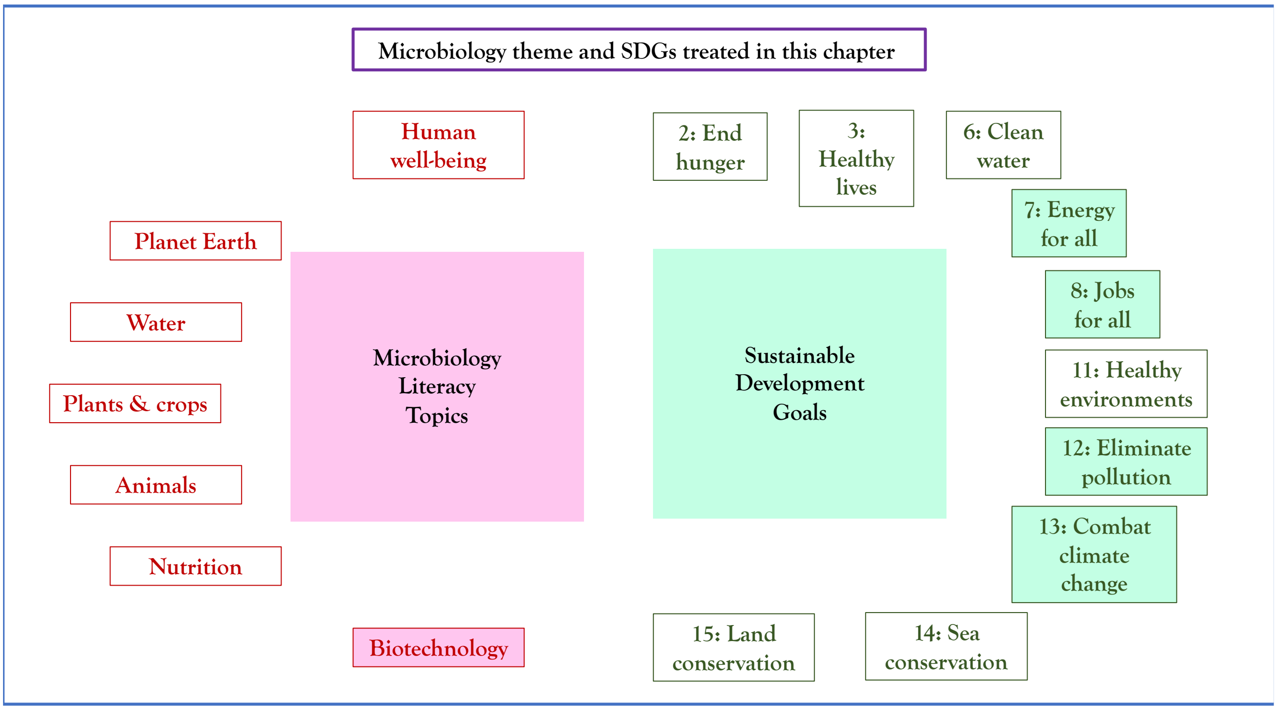
Storyline

Much of the energy being used to power our lives comes from fossil fuels such as coal, natural gas and petroleum. These energy sources are non-renewable, are being exhausted, and also pollute the air, water and soil with toxic chemicals. Their mining, transportation, refining and use are associated with a large carbon footprint that contributes significantly to global warming. Replacement of fossil fuels by clean, renewable forms of energy is paramount to creating a sustainable and healthy future. Microbes are able to produce from plant materials biofuels – compounds that are identical or equivalent to compounds in fossil fuels. Development of the biofuel industry will enable us to obtain energy from renewable sources and will create new, high-quality rural jobs. Bioethanol and biodiesel are currently in the market, but advances towards more sophisticated fuels are underway.

The Microbiology and Societal Context

The microbiology: microbial conversion of plant materials to bioalcohols, biodiesel, biogas. *Sustainability issues:* renewable energy; energy security; greenhouse gas production/global warming; environmental pollution; rural employment stability.



Using Microbes to make Biofuels: the Microbiology

1. ***The need for renewable fuels.*** The industrial revolution at the end of the 19th century sparked the beginning of the modern era. Throughout the 20th century and until now, huge economic and technological leaps have been made. Improvements in the production of goods and advances in medicine have contributed to increases in life expectancy across the world. The increase in human population, combined with the development and expansion of terrestrial, maritime and air transportation have led to a highly connected world. Combined, these changes have led to an enormous increase in the demand for energy.

With bans on the use of nuclear energy being enacted in some countries, due to short- and long-term safety concerns, and nations working to reduce reliance on fossil fuels (i.e., petroleum, coal, natural gas, etc.) due to their polluting effects, we are faced with the need to develop other ways of creating energy. These alternative sources of energy can be sourced from sunlight (i.e., photovoltaic and thermosolar), wind, ocean tides and microbes. No single means of making energy is sufficient to cover the world's energy demands; however, when developed and used concurrently, these approaches may be sufficient to meet current and future demands.

2. ***Biofuels.*** The Kyoto Protocols and the most recent Paris Climate Agreement call for the use of clean, green and renewable transportation fuels to replace gasoline, diesel and jet fuel. Biofuels are fuels produced from biological materials, most often cereal grains, sugarcane or biomass derived from plants or wastes, and they represent a promising alternative to fossil fuels. Biofuels are considered renewable fuels because they are derived from plant materials that are made from CO_2 fixation through photosynthesis that is powered by sunlight: plants can be grown continually to provide a constant supply in contrast to the constantly diminishing supply of fossil fuel materials.

A number of biofuel programs have been implemented in the United States, Brazil and the European Union with the aim of not only reducing emissions, but also reducing the importation of fossil fuels and enhancing the security of national fuel supplies. Biofuels offer a number of social, economic, environmental and technical benefits, which include: moderating oil prices, creating rural jobs, reducing global carbon emissions and decreasing soil erosion. That said, controversies have arisen, such as the fear that use of agricultural land for biofuel production will endanger food production (the "food versus fuel" debate).

3. ***Biofuels and emissions.*** While the use of biofuels for motor vehicles significantly reduces net emissions it has been proposed that, in order for a biofuel to be a viable fossil-fuel alternative, it should

   a. provide a net energy gain,
   b. have environmental benefits,
   c. be economically competitive, and
   d. be produced in large amounts without reducing food supplies.

Because plants use CO_2 to grow, and CO_2 is a greenhouse gas, they reduce net emissions of greenhouse gases, thereby fulfilling condition b. However, for a specific biofuel to be carbon neutral, the total carbon sequestered by the plant must compensate for all the emissions linked to the production and manufacture of a given biofuel. These emissions include those caused by direct or indirect changes in land use, the amount of carbon sequestered and the amount of greenhouse gases emitted. In general, achieving carbon neutrality for biofuels requires high plant yields and low emissions.

Despite the potential represented by biofuels, current estimates indicate that only about 1% of the energy used globally can be traced back to a biofuel source. Therefore, there exists great opportunities to increase the use of renewable fuels. More recent data suggests that, in certain sectors, biofuel use is gaining traction: data from 2018 estimates that worldwide biofuel production reached 152 billion liters (40 billion gallons US) and provides about 3% of the world's fuel for road transport. In order to reduce dependency on petroleum, several international agencies and governments are aiming to use biofuels to supply 25% of their transportation energy by 2050. In this Topic Framework, we will focus on the fundamental concepts underlying the production of biofuels using microbes.

4. ***Crops for biofuels.*** Energy crop plants are grown specifically for biofuel production. They vary according to geography: for example, corn, soybeans, willows and switchgrass are common energy crops in the United States; rapeseed, wheat, sugar beet and willows are preferentially grown in northern Europe; sugarcane is grown in Brazil; palm oil and *Miscanthus giganteus* (giant silver grass) are grown in Southeast Asia; and sorghum and cassava are grown in China. Worldwide, corn grain and sugarcane are the most common biofuel crops, while *Miscanthus* is believed to be the most efficient biofuel crop. In addition to plant material, algae and the organic fraction of municipal solid waste are also considered biological feedstocks for biofuel production.

Depending on the source of the biological material, biofuels are referred to as either first, second, third or fourth generation. First-generation biofuels are profitable in the United States, but less so in Europe; second-generation biofuels require governmental subsidies; and third- and fourth-generation biofuels are currently not considered to be profitable.

5. ***First generation (1G) biofuels.*** First-generation biofuels are conventional biofuels made from food crops grown on arable land. Plants can be grown explicitly for the production of biofuel. The sugar, starch or vegetable oil obtained from the crops is converted into biodiesel or ethanol. This can occur via transesterification, or via fermentation mediated by yeast or bacteria. The impetus to bring first-generation biofuels to the market was driven by the perception that they reduce climate gas emissions, and also by factors such as oil price spikes and the need for increased energy security.

However, first-generation biofuels became marred in controversy because, as competitors for arable land, they were soon perceived as a threat to agricultural food production and food security. Pollution associated with first-generation biofuels also became a point of contention, especially after the Nobel-prize winning chemist Paul J. Crutzen published findings indicating that emissions of nitrous oxide (N_2O) – a powerful greenhouse gas – made during the production of biofuels may contribute more to global warming than the amount of CO_2 fixed.

To overcome these challenges, the industry has focused on the development of biofuels using alternative sources of biological materials and new innovative technologies, which have given rise to second-generation biofuels.

6. ***Second generation (2G) biofuels.*** Second-generation biofuels are made from lignocellulosic biomass or woody crops, agricultural waste, and the organic fraction of municipal solid waste. Therefore, the feedstocks used to generate second-generation biofuels are either by-products of other crops (i.e., food crops on arable land), or are grown on land that cannot be used to effectively grow food crops.

The selection of land on which to grow the feedstock is a critical determinant of the sustainability of the biofuel, and a key consideration is minimization of the competition between land use for biofuel production and food production. In general, second-generation biofuels avoid this competition and do not use extra water or fertilizer.

The use of municipal and household waste for biofuel production is an emerging approach—one that makes use of biomass that is currently a largely unused resource. Use of this biomass as an energy source has the potential to improve waste management, fuel security and help address climate change.

7. ***Third generation (3G) biofuels.*** Third-generation biofuel is produced using algae. This involves growing algae with a naturally high oil content in ponds of wastewater treatment plants. The oil-rich algae are then harvested and processed into biofuels. At present, due to complex processing requirements and drops in fuel prices, these biofuels are not cost-competitive and are not expected to become competitive in the near to midterm.

The advantage of algaculture is that it does not compete with food production because it requires neither farmland nor fresh water. One approach involves the production of hydrogen and ammonia using photosynthetic microbes such as cyanobacteria like *Anabaena*, or bacteria of the genus *Rhodopseudomonas*. In general, the overall yields for third-generation biofuels are limited by light-to-energy conversion efficiencies and the concentrations of the products produced. While hydrogen produced from water represents the cleanest fuel in existence, before third-generation biofuels become feasible, detailed techno-economic analyses are required to define the conditions required to achieve profitable industrial-level production.

8. ***Fourth generation (4G) biofuels.*** Fourth-generation biofuels include electro-fuels and photobiological solar fuels. At present, these fuels are not considered to be a realistic source of energy. Microbial fuel cells convert the chemical energy stored in wastewater or soil into electrical energy via metabolic processes present within electrogenic microorganisms. The power generation capability of this technology is not currently viable from an economic perspective.

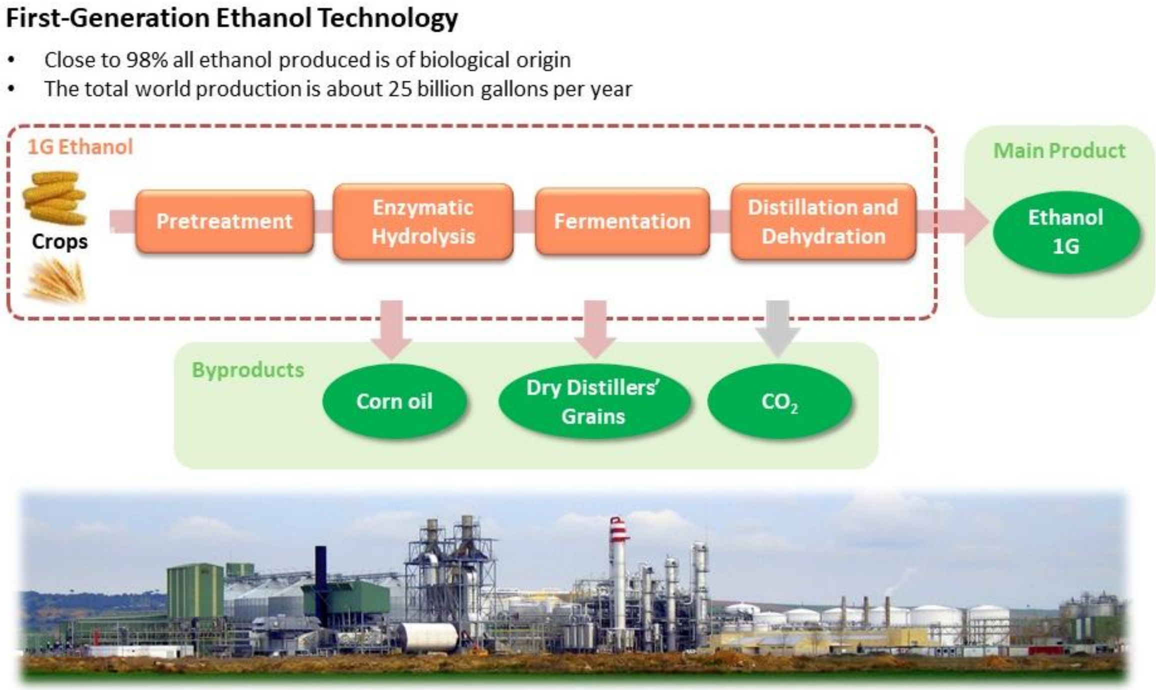
9. **Types of biofuels.** All biofuels can be made as liquids (alcohols, biodiesel) or gas (biogas). Below, we describe a selection of the current fuels in use.

a. Alcohol biofuels

i. <u>Bioethanol.</u>

Currently, bioethanol is the most relevant biologically produced commodity. Almost all of the ethanol used in the world for pharma, solvent industries and fuels is produced through biological fermentation. Bioethanol is a 1G fuel that is a commonly produced worldwide, particularly in Brazil and the United States. Alcohol fuels are produced through the fermentation of sugars derived mainly from corn grain and sugarcane, as well as from sugar beet, wheat grain (or other cereal grains), molasses and various other plants, including fruit and fruit waste. Figure 1 summarizes the process with cereal grains.

In the case of grain, the first step of biofuel production is the hydrolysis of starch using amylases. This process produces simple sugars – mainly glucose – which are then fermented to ethanol using microorganisms such as yeasts or bacteria (e.g., *Zymomonas*). In the United States, ethanol production rates are in the range of 14 –15 billion gallons per year at corn dry mills. These mills produce not only ethanol but also corn oil and dry distillers' grains, which are used as animal feed. The CO_2 produced during fermentation is harvested and used for carbonated drinks or for medical uses. This first-generation ethanol technology is quite mature and industrial ethanol plants are usually profitable in the United States. In Brazil, around 5 billion gallons of ethanol are produced annually and the leftover waste (i.e., bagasse) is often burnt in the mills to generate extra energy.

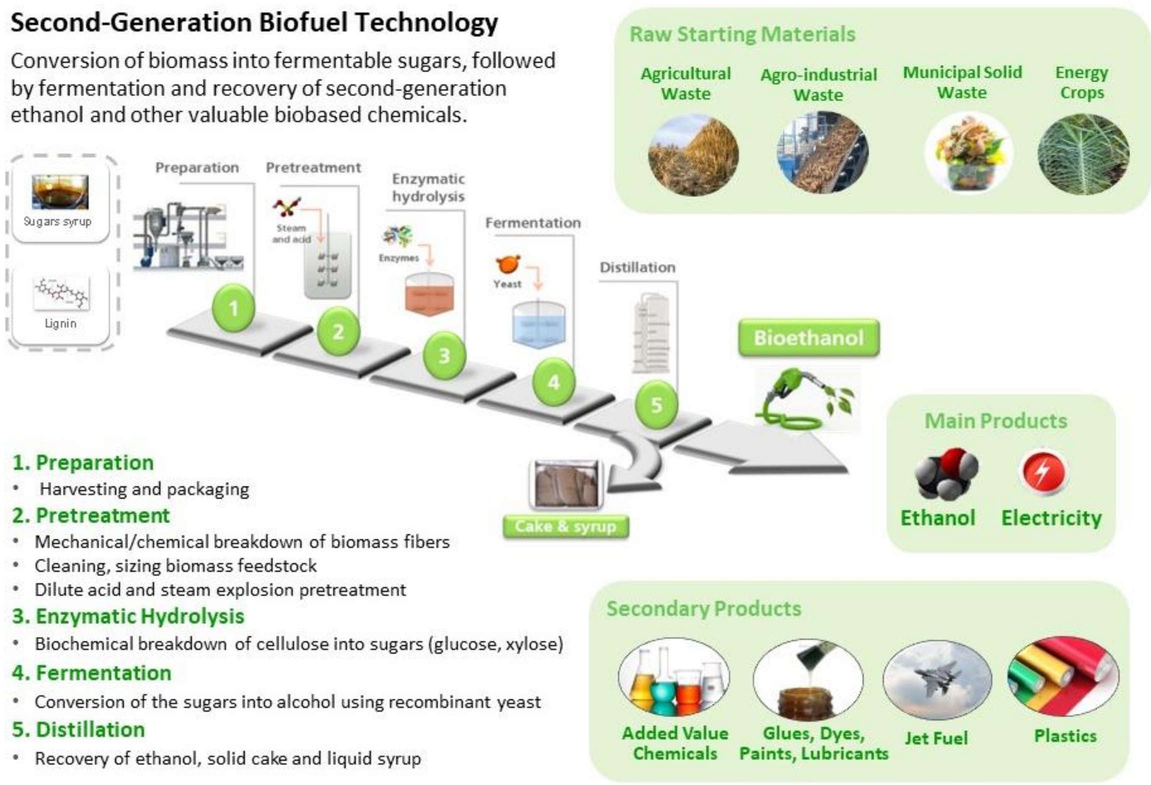


Scheme showing the steps in the production of 1G ethanol from grain. Hot water is used to generate a corn mash, which is subsequently subjected to enzymatic hydrolysis with amylases, which release sugars from starch. Glucose is then fermented by yeast or bacteria to produce ethanol that is eventually distilled and dehydrated for commercial use. In addition to ethanol, 1G factories produce corn oil, harvest CO_2 for industrial and medicinal uses, and generate a solid substrate called 'dried distillers grain with solubles', which is an excellent feed for ruminants. The picture at the bottom depicts the 1G ethanol plant of BCyL in Salamanca, Spain.

Ethanol can be used as a fuel for vehicles in its pure form (E100), but it is usually used as a gasoline additive to increase octane rate and decrease vehicle emissions. Modern car petrol engines can run on blends of up to 10% (v/v) bioethanol with gasoline (E10); however, it should be noted that ethanol has a smaller energy density than gasoline. For this reason, it takes more fuel (volume and mass) to produce the same amount of work.

An advantage of ethanol is that it has a higher-octane rating than ethanol-free gasoline, which allows an increase of an engine's compression ratio and increased thermal efficiency. It has been proven that adding 10% (v/v) ethanol to gasoline reduces the emission of fine particulate matter by 36%, and by as much as 65% in cars with large displacement volumes.

Through an attempt to address the "food versus fuel" controversy, the biofuel industry searched for new feedstock sources. This was necessary not only to address the above issue, but also because it was calculated that if all the corn in the United States was used to produce biofuels, it would only satisfy 12% of the demand for gasoline (Hill et al., 2006). Thus, the industry has begun looking at using agricultural waste left over after harvest as a source material for bioethanol production.



Scheme showing the steps from biomass to bioethanol production. Plants for 2G ethanol carry out three key steps (i.e. steps 2, 3 and 4 in the upper left side of the scheme). The three core areas correspond to the processes developed by Abengoa Bioenergy. In addition to ethanol, 2G plants produce high quality lignin and different syrups that can be transformed into added value chemicals.

The production of 2G bioethanol usually requires three major steps: i) the physicochemical pre-treatment of the biomass, to make the polysaccharides (cellulose and hemicellulose) in lignocellulosic material accessible to the cellulases and hemicellulases used to convert complex sugars into simple sugars; ii) the enzymatic breakdown of biomass components (i.e., cellulose and hemicellulose) into constituent sugars; and iii) fermentation using specialized yeasts.

At present, the main hurdles facing 2G ethanol seem to arise from mechanical issues in the handling of materials and the efficient operation of the pre-treatment units. Another relevant hurdle is the price of the required enzymatic cocktails; these at present cost more than amylases by an order of magnitude. The main source of these cocktails are enzymes secreted by fungi, which use them to grow on plant material, like leaf litter, dead wood, etc., by metabolizing lignocellulosic residues. These enzymatic cocktails can enable the release >80% of the monosaccharide sugars that are present in celluloses and hemicelluloses. Most of the sugar used to produce ethanol in 1G processes is glucose. While glucose is also the predominant sugar in 2G processes (approximately 75% of total sugars), significant amounts of other sugars are also involved, including xylose (23%) and arabinose. Fermentation of sugars released from corn stover, bagasse and other agricultural residues requires the use of specialized yeasts that are able to simultaneously ferment glucose and xylose.The yeasts used in 2G fermentations are genetically modified to convert more than 96% of glucose and more than 90% of xylose into ethanol with overall fermentation yields >90% of the theoretical maximum—an achievement that demonstrates how far this technology has progressed.

In terms of availability, forest wood has the potential to serve as major sources of non-food biofuel feedstocks, and this availability has started to attract attention as a global renewable resource of fibre for ethanol production. This is particularly so when trees for energy crops are grown in marginal soils that are not able to support agriculture. Among various forest woods, willow trees have demonstrated high potential for use in biofuel production, because they produce large quantities of accessible sugar, are fast-growing, and can tolerate harsh environmental conditions, such as windy slopes.

An industry for the production of cellulosic ethanol from agricultural waste is also beginning to gain momentum. This approach offers significant opportunities for a range of players, including farmers, biotechnology firms, project developers and investors. However, at present this sector is still in an early phase, and requires significant subsidies before it can mature.

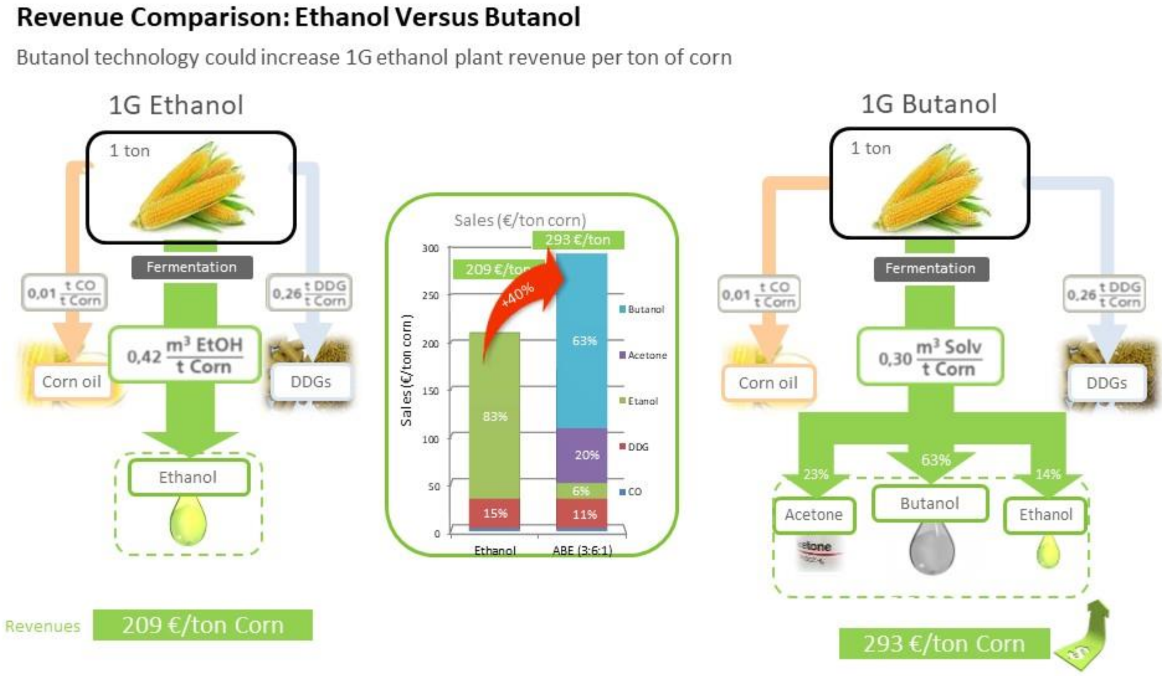
Another source of cellulosic material is the organic fraction recovered from municipal solid waste (MSW). The technologies used in these processes are very similar to that used for production of ethanol from corn stover or bagasse, with the added requirement for a series of early steps to separate the organic fraction from other materials present in the waste biomass.

A study by Turner and colleagues (2015) stressed the importance of ensuring that MSW is sustainably sourced. If MSW is properly sourced, its use could reduce greenhouse gas emissions by 65%, even when considering all possible indirect emissions. In the United States, the organic fraction of MSW is about 61% according to the EPA. If the 164 million tons that are currently diverted to landfills were converted to bioethanol, about 7.5 billion gallons of ethanol could be produced – the equivalent of around 250 million barrels of petrol. Furthermore, it has been estimated that biofuels from MSWs and agricultural residues could replace 16% of fuel used by the United States transportation sector by 2030. In looking towards 2050, there is great potential for the production of biofuels from non-edible plant materials and MSW residues.

ii. Biobutanol.

Biobutanol (also called biogasoline) is often cited as a potential replacement for gasoline because it can be used directly in internal combustion engines. In the past, butanol was produced through what is known as ABE fermentation, an anaerobic process efficiently carried out by a number of strains of the genus *Clostridium* that yield a mixture of acetone, butanol, ethanol at a ratio of 1:6:1. Although ABE fermentation has since been replaced by the chemical production of butanol from petrol, a number of research efforts have been initiated to increase the proportion of butanol that can be produced through fermentation – efforts that have led to processes capable of yielding ABE ratio of 1:8:1. These gains have been achieved through the development of *Clostridium* strains with a genetically modified metabolic pathway, as well as through selecting strains that are able to withstand higher butanol concentrations. With further research, technical advancements and industry investments, biobutanol has the potential to become more profitable than ethanol. Moreover, a number of clostridia strains are able to degrade lignocellulose material (i.e., from agricultural waste, forestry residues, etc.), suggesting that a second-generation biobutanol industry is feasible.

Those who support a shift to butanol production point to three key benefits: (i) butanol has a higher fuel density than ethanol and is less corrosive; (ii) it can be added to gasoline at a higher blend ratio, the so called BUT16; and (iii) it is highly compatible with existing petroleum distribution systems, including fuel pumps. However, butanol also has some disadvantages in comparison to ethanol in having has a lower octane rating, and a lower heat of evaporation.



Scheme comparing bioethanol and biobutanol production. The values given correspond to the processing of 1 tonne of corn grain. Biobutanol has a number of social and environmental advantages over bioethanol, and is economically more profitable.

b. Biodiesel

Biodiesel, another transportation fuel, can be produced from leftover food products. Biodiesel is the most common biofuel in Europe and is generated through a process the involves transesterification of vegetable oils or animal fats. It can be used as a fuel for vehicles in its pure form (B100), and because biodiesel is an oxygenated fuel and has a higher hydrogen and oxygen content than standard diesel, its combustion leads to lower particulate and carbon monoxide emissions. However, using pure biodiesel may increase emissions of the greenhouse gas nitrous oxide. Biodiesel is mainly produced from vegetable oils and microbes play only a minor role in the process. Nonetheless it should be mentioned that several fungi accumulate large amounts of fatty acids that are easily converted into alkanes. One of these oleaginous fungi is a strain of *Aspergillus* that is able to produce 20% more fatty acids than normal fungi; some strains of this fungus are able to make fatty acids directly from free sugars and cellulosic materials.

c. Biogas

Biodegradable outputs from industry, agriculture, forestry and households can be used for biofuel production through anaerobic digestion to produce biogas. Anaerobic biogas production is catalyzed by methanogens, which digest material inside a closed system, known as anaerobic digester, biodigester or a bioreactor. Biogas is primarily methane (CH_4) and carbon dioxide (CO_2), and methane can be combusted or oxidized with oxygen, releasing energy. Advanced waste treatment technologies can produce biogas comprising 55%–75% methane. Reactors with free liquids can produce gas comprising 80%–90% methane using *in situ* gas purification techniques. Biogas is a renewable energy source because its production and use cycle is continuous, and the process generates no net carbon dioxide. Furthermore, the solid by-product, which is known as digestate, can be used as a fertilizer. Biogas can also be compressed after removal of carbon dioxide and used to power motor vehicles; and it can be cleaned and upgraded to meet natural gas standards.

Relevance for Sustainable Development Goals and Grand Challenges

Biofuels represent a set of renewable fuels that contribute to replacement of fossil fuels. Although controversies such as food versus fuel are of concern, combustion of biofuels is cleaner than fossil fuels and also serves to reduce the emission of toxic chemicals. Biofuels also promote the use of land and marginal lands to grow energy crops, which leads to the creation of high quality and stable rural jobs. Bioethanol and biodiesel are currently players in the market and have the capacity to replace gasoline and diesel in combustion motors.

The value of biofuels goes beyond their use as transportation fuels, and attention should be given to the economic and environmental benefits of the co-products of biofuels. Both 1G and 2G biofuel industries serve to significantly reduce greenhouse gas emissions, diminish our reliance on crude oil, encourage energy diversity and promote the creation of rural jobs. Presently, the long-term success of 2G ethanol requires financial incentives and supportive regulations, which are instrumental for driving the commercial production and adoption of advanced biofuels.

The field of biofuel research is an exceptionally dynamic and exciting arena that has the potential to transform how we produce energy. It holds the key to creating a more sustainable and circular economy, and relies on starting materials that are currently considered waste. There is an enormous impetus for the development of affordable genomics technologies, and these are going to be critical for next generation fuels. The revolution in synthetic biology is enabling the development of novel biofuels capable of replacing kerosene. These new fuels will help ameliorate CO_2 emissions from a variety of human activities, including transportation and air flight. Biofuels are highly aligned with UN SDG targets because they have the potential to reduce greenhouse gas emissions, reduce pollution, promoting energy security, and create stable and high-quality rural jobs.

As part of the UN's global development agenda, and as outlined in the latest summit outcome document, SDGs serve as a cornerstone initiative until 2030. While SDGs are not legally binding treaties, their realization is driven by moral and political commitments. One of these agreements compels UN countries to move towards the responsible use of energies and the replacement of fossil fuels by green renewable sources such as biofuels.

The Evidence Base, Further Reading and Teaching Aids

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