Marcos Silveira Buckeridge
Gustavo H. Goldman *Editors*

Routes to Cellulosic Ethanol

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Introduction

This book is a result of one of the workshops organized by the BioEn, the Bioenergy Program of the Foundation for Advancement of Science in the State of São Paulo (FAPESP). The BioEn was established in 2009 aiming to bring together the research in bioenergy at São Paulo State, which is the second largest producer of sugarcane in Brazil and one of the largest producers of bioethanol in the world in 2010.

This book is also a product of the National Institute of Science and Technology of Bioethanol (INCT-Bioetanol), presenting some of the results of its associated laboratories and collaborators.

Brazil and US are presently the largest producers of bioethanol on Earth and motivated by the growing effect of the global climatic changes and also energy security, both countries are focusing on increasing even more the production of this important liquid biofuel for economical reasons too.

The obvious way to do that using biomass feedstocks is to learn how to extract energy from the cell walls as they form up to 70% of the plant body. The valuable polymers composed of carbohydrates linked by glycosidic linkages are either left in the field for microorganisms to use them or are used for production of electricity (in the case of sugarcane in Brazil) in a not so efficient way.

There is a lot to learn and the biological sciences are now in an excellent position to provide valuable information that can lead us to potentially double the production of bioethanol.

However, reaching this goal is not a trivial task. As will be seen in the chapters of this book, the main targets are related to aspects concerning how to control the architecture of the plant cell walls by modifying plant genome for instance and at the same time to find microorganisms that are able to degrade the cell walls efficiently and produce free sugars that can be fermented by yeast. In order to do that, one needs to learn also about enzyme structure and how enzymes interact with carbohydrate substrates.

Microorganisms have the potential to be redesigned by molecular biology techniques and soon by synthetic biology, so that efficient enzyme cocktails can be produced and introduced commercially. Also, yeast will have to be taught how to use pentoses, along with hexoses, in order to produce ethanol.

The process of bioethanol production from biomass feedstocks such as maize, sugarcane and miscanthus, eucalyptus, and others will have to include also the agronomical dimension of the problem that will have to be connected to the industrial processing. However, in this book the agronomical side of the story is not visited.

In this book, some chapters deal with bioenergy in general, comparing the energy matrices of US and Brazil and also comparing different forms to produce bioenergy, such as gasification, pyrolysis, and biodiesel from oils. However, the main focus is on different aspects that are important to reach better ways to deconstruct biomass, i.e., cell walls.

However, we did not forget to include information about the thermal route, because we believe that all means of science have to be applied in order to increase the production of renewable energy to cope with the enormous challenges that humanity is facing in this century.

We hope that this book will be a contribution to help this part of science and technology to advance.

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Part I
Bioenergy

Chapter 1
The Role of Biomass in the World's Energy System

Jose Goldemberg

1 Introduction

Since the dawn of civilization until the middle of the nineteenth century, biomass was the world's dominant source of energy and its consumption grew from approximately 50 million tons of oil equivalent in the beginning of the Christian era to 1,000 tons of oil equivalent today (a 20-fold increase). In this period, biomass has supplied the needs of the population for cooking and heating as well as shipbuilding, housing, and forges to process metals (mainly for weapons). Presently, biomass accounts for about 10% of the world's primary energy consumption. The other 90% is made up of nonrenewable fossil fuels (80%), hydroelectricity (2%), nuclear energy (6%), and renewable solar energies (2%) (Fig. 1).

The fraction of biomass used varies widely across different regions of the globe. It is as low as 3.9% in the OECD countries, 18.8% in all the developing countries as a whole, and it reaches 61.5% in sub-Saharan Africa (Table 1).

Such uses, in many cases, have led to a reduction of the forest cover of countries and regions of the world. This was pointed out as early as 400 BC by Plato when mourning the lost forests described by Homer that covered the barren hills of Greece centuries ago. As a whole, there was a reduction of 7.01 million square kilometers in total world's forest area since preagricultural times to the present, mostly for food production, although the contributions of energy use to such reduction are not negligible, particularly in Africa and Latin America and the Caribbean.

With the large increase in population since 1500 CA and particularly after the end of the eighteenth century with the development of the Watt machine, coal started to replace biomass. In the twentieth century, oil and gas entered the scene and contributed decisively to replace coal as well as biomass as can be seen in Fig. 2.

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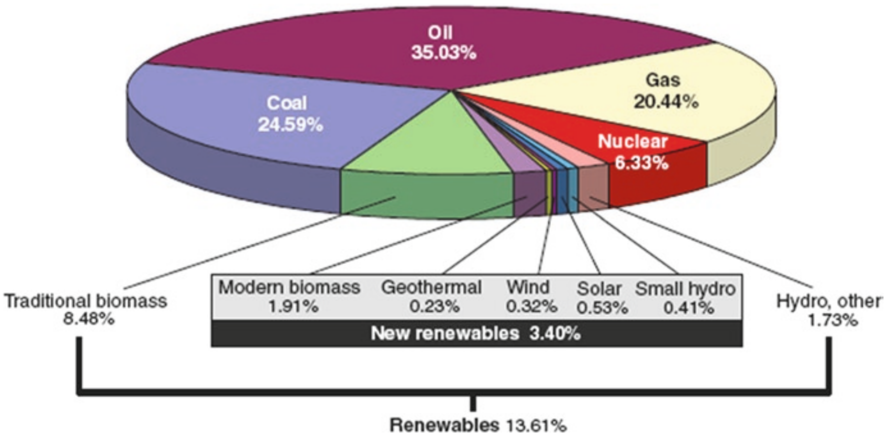

Fig. 1 World total primary energy supply 2004, shares of 11.2 billion tons of equivalent, or 470 EJ (Goldemberg 2007)

Box 1 Definition of Biomass (Goldemberg and Coelho 2004)

Biomass (plant matter) is usually classified into two categories:

i. "Traditional biomass," which is used in inefficient ways such as the highly pollutant primitive cooking stoves used by poor rural populations, leading to deforestation in many cases.
ii. "Modern biomass," which refers to biomass produced in a sustainable way and used for electricity generation, heat production, and transportation of liquid fuels. It includes wood and forest residues from reforestation and/or sustainable management as well as rural (animal and agricultural) and urban residues (including solid waste and liquid effluents).

The International Energy Agency (IEA) uses somewhat different definitions, "Combustible renewables and waste comprises solid biomass, liquid biomass, biogas, industrial waste and municipal waste. Biomass is defined as any plant matter used directly as fuel or converted into fuels (e.g., charcoal) or electricity and/or heat. Included here are wood, vegetal waste (including wood waste and crops used for energy production), ethanol, animal materials/wastes and sulphite lyes (...) also known as 'black liquor (...)'. Municipal wastes comprises wastes produced by residential, commercial and public service sectors that are collected by local authorities for disposal in a central location for the production of heat and/or power. Hospital waste is included in this category." The agency also recognizes that "Data under this heading are often based on small sample surveys or other incomplete information." The available statistics do not separate unsustainable sources of biomass

(continued)

Box 1 (continued)

(e.g., fuelwood from deforestation) from the sustainable (e.g., biodiesel). Until more comprehensive data are published for all countries, it could be assumed that all combustible renewables and waste (CRW) from developed countries are renewable; for developing countries, at least, the CRW applied into electricity production (thus a modern process) can also be considered renewable.

Source Renewable energy–traditional biomass vs. modern biomass" Goldemberg J. T. Coelho, Suani–Energy Policy 32 Nº 6 pp. 711-714, 2004

2 Energy and Transportation

The main reason for that was the fact that in the twentieth century, road transportation became one of the most significant consumers of oil products. Today, transport represents 22% of total energy consumption in industrialized countries and 14% in the developing countries. About half the world's oil production is consumed by road vehicles. The fleet's annual increase is about 10 million automobiles (doubling every 20 years or so) and five million buses and trucks worldwide (Goldemberg 1998). If the trend continues, a billion vehicles will use the world's roads by 2030. Not only is the number of automobiles growing but there is also a tendency to drive more, so the number of vehicle-miles traveled is increasing rapidly in countries such as the US.

The heavy dependence of transportation on oil is not a sustainable situation because of the problems associated with such resource:

1. Exhaustion of resources, which are estimated to last approximately 40 years with presently available technologies.
2. Security of supply, which is frequently threatened since most of oil used today comes from politically unstable regions (particularly the Middle East).
3. Environmental impacts, which can be local, regional, and global.

3 Environmental Impacts

Environmental impacts, particularly global ones, are presently becoming an overriding concern due to their impacts in climate change in contrast to local and regional impacts, which are already well known and being addressed by governments.

- *Local impacts* are mostly felt in cities such as Bangkok, Mexico City, Los Angeles, and Athens during peak traffic periods. At these times, air pollution in the city can approach crisis proportions and seriously affect the local population.

Table 1 Fractions of biomass in different regions of the world (2005) (International Energy Agency)

	Biomass							
	Municipal waste [a]	Industrial waste	Primary solid biomass[b]	Biogas	Liquid biofuels	Total biomass	Total primary energy supply (TPES)	Share of biomass in World TPES
	TJ	TJ	TJ	TJ	TJ	TJ	TJ	(%)
OCDE	832,261	271,863	6,142,592	376,773	725,782	8,349,271	232,266,749	1.77
Europe non-OCDE	8	6,588	254,458	390	0	261,444	4,396,935	0.06
Latin America	0	0	3,438,412	0	308,476	3,746,888	20,952,045	0.79
Asia	37,994	0	23,123,472	143,186	6,601	23,311,253	126,494,365	4.94
Africa	0	0	12,019,416	0	0	12,019,416	25,345,589	2.55
Middle East	0	0	43,052	0	0	43,052	21,073,714	0.01
Former USSR	315	149,204	352,462	569	328	502,878	41,036,418	0.11
World	870,578	427,655	45,373,856	520,918	1,041,187	48,234,194	471,565,815	10.23

[a] Municipal waste: the split for renewable and nonrenewable waste is also available
[b] Primary solid biomass: data are also available for charcoal

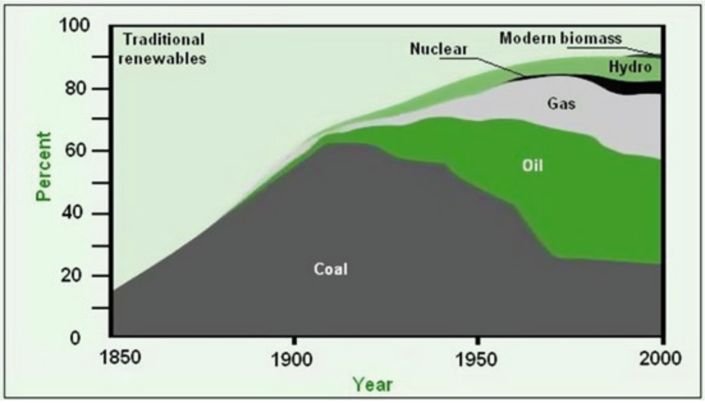

Fig. 2 (World Energy Assessment 2000)

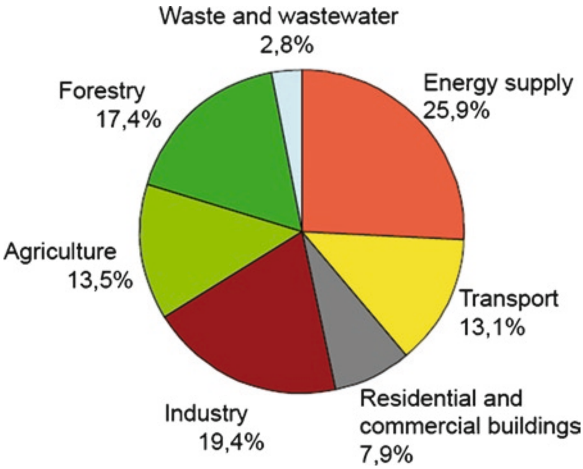

Fig. 3 GHG emission by sector in 2004 (IPCC 2007)

The problem is sometimes aggravated by a combination of local topographical and meteorological conditions that trap pollutants near the ground for extended periods of time.

- *Regional impacts* are mainly due to acid rain which is caused by nitrogen oxides emissions from the transport sector. The emissions from the increasing numbers of aircraft are estimated to total around three million tons annually (equivalent to about 15% of present automobile NOx emissions). In contrast to near ground-level emissions, where the nitrogen oxides are usually washed out by rain within days (generating acid rain), they persist in the upper atmosphere for long periods, contributing to ozone destruction.
- *Global impacts* are mainly due to the global fleet of motor vehicles, which is presently responsible for 13.1 of greenhouse gas emissions and 19.2% of the world's CO_2 output (Fig. 3).

4 Strategies to Face the Impacts of Transportation

There are three strategies to reduce the dependence of transportation sector oil:

1. Systems operation improvement
2. Technical approaches
3. Alternative fuels

- *System operation* includes shifting passengers and freight to transport modes that result in lower consumption and consequently lower emissions of pollutants and CO_2. Other measures include driving habits such as sharing and several restrictions on circulations of vehicles in problematic areas such as central portions of large cities as it was done in London.
- *Technical approaches* involve

  - *Engine efficiency improvement* – increasing effectiveness with which the fuel energy is converted into useful work for powering the automobile. Engine efficiency is the product of two factors: *Thermal efficiency*, expressing how much of the fuel energy is converted into work to drive the engine and vehicle and *Mechanical efficiency*, the fraction of work that is delivered by the engine to the vehicle.

- *Alternatives fuels* to gasoline for Otto-cycle automobiles and diesel for Diesel-cycle trucks

  - *Liquefied petroleum gas* (LPG) and compressed natural gas (CNG) have a higher hydrogen-to-carbon ratio than gasoline, thereby emitting less CO_2 per unit of energy. They have a higher octane number than gasoline, permitting the use of higher compression ratio engines. No major infrastructure changes are required for LPG or CNG use.
  - *Hydrogen* can fuel ultra-low-emission vehicles. Storage is a problem due to its low energy density. Compressed hydrogen storage is the most probable scheme, though liquid hydrogen or metal hydride storage is also possible.
  - *Biofuels* include ethanol produced from sugars and starch by fermentation with yeasts. Ethanol can be used pure or as a gasoline extender in spark-ignition engines. In addition, lignocellulose – from energy forestry, agricultural and forest industry residues, and the carbohydrate fraction of municipal solid waste (MSW) – is a further source of biomass liquids. Such a resource is 20 times more plentiful in the US than maize, and does not compete with food production (Fig. 4).

Box 2 Electrical Vehicles

Electric vehicles, using batteries, are of great interest today, especially as urban vehicles. If the electricity that fuels them comes from a nonfossil source, they can yield a significant greenhouse gas emission reduction. The key barrier to their implementation is the current state of chemical battery technology, resulting in high costs, heavy automobiles, and limited range. Also, while a gasoline automobile can be fueled in a few minutes, electric automobiles are generally fueled much more slowly over a time span of hours. Large-scale introduction of electric vehicles could require major infrastructure changes, not only in the energy distribution system and the automobile itself, but also in the electric power generation industry.

Fuel cells produce power electrochemically as opposed to combustion processes in conventional engines and can potentially reach significantly higher conversion efficiencies – perhaps by a factor of 2–3 – compared to today's internal combustion engine. Fuel cells come in several varieties, but the proton-exchange-membrane (also called solid polymer) fuel cell is the leading candidate for automobiles because of cost, size, simple design, and low temperature (>120°C) operation. The technology was originally used in the US space program. The fuel cells require hydrogen fuel, which may be generated on-board the automobile by reforming methanol or natural gas.

Source Inter Academy Council (2007)

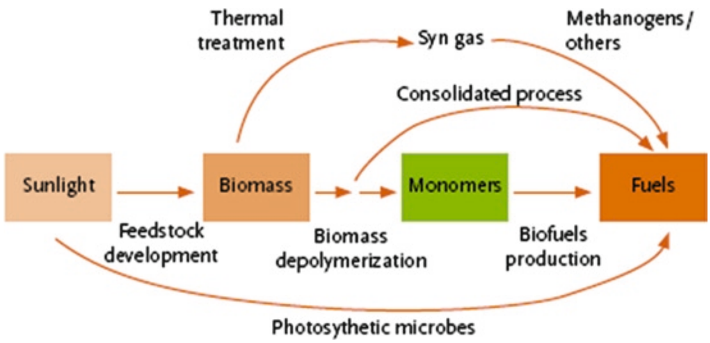

Fig. 4 (InterAcademy Council 2007)

5 Biodiesel and Ethanol

A number of plant-derived oils have also been considered for possible use as fuels in diesel engines including sunflower, soya, groundnut, cottonseed, rapeseed, palm oil, and castor oil. Vegetable oils have been tried unsuccessfully in the past, raising problems of carbon deposits in the engine, clogged injection systems, high particulate emissions, reduced efficiency, and high maintenance needs. Diesel engines operating on these fuels have reduced efficiency and higher maintenance requirements.

Biodiesel oil is a potentially important enhancer or replacer of conventional diesel fuel. It can be prepared from many renewable raw materials that include soybean, rapeseed, and palm oils. The viscous, high-boiling triglycerides are processed to obtain more volatile methyl esters of the straight-chain fatty acids. Biodiesel oil is in the early stages of development, but specimens of it have undergone many successful long-term tests in buses, trucks, and tractors. In some of the tests, a mixture containing 80% conventional fuel and 20% biodiesel oil has been employed. Tests using 100% renewable fuel have also been successful. In both instances, the results were superior in many ways to those noted when conventional diesel fuel was employed. The renewable fuel is practically sulfur-free. It is non-toxic and quickly biodegradable if spilled. On combustion, it produces less toxic particulate matter. Only minor adjustments of existing engines are required to attain optimum performance.

Of all these approaches, the use of ethanol is the one that has reached maturity and is making a real contribution in reducing gasoline and diesel oil consumptions.

Production of ethanol to supply the needs of this fleet takes place in 405 distilleries, most of which are equipped for the dual production of sugar and ethanol. In 2007, production reached 22 billion liters. For 2008, the expected production was 26.1 billion liters and assuming a growth of 8% per year – which took place in the last few years – it should reach 30.5 billion liters in 2010 using approximately an area of four million hectares of sugarcane. There are at present 35 new distilleries starting production in 2008/2009 and another 43 in various degrees of implementation. In 2015, production should reach 47 billion liters and the land required approximately six million hectares (Goldemberg and Guardabassi 2008).

The cost of production of ethanol in Brazil dropped significantly over the years as seen in Fig. 5.

In 1980, it was roughly three times the price of gasoline in the international market, but it became competitive with gasoline in 2004 due to technological gains and economies of scale. Productivity increases of almost 4% per year in the last 30 years took place. The number of liters of ethanol per hectare of sugarcane increased from 3,000 liters per hectare to more than 6,000 liters per hectare. Ethanol is today fully competitive with gasoline without any subsidies (Goldemberg et al. 2004).

The drivers for such extraordinary expansion of ethanol production from sugarcane were not only economic and strategic – to reduce dependence from petroleum imports – but also environmental.

Ethanol does not have the impurities that come along with gasoline such as sulfur oxides and particulates, which are the main cause of the bad quality of the air in

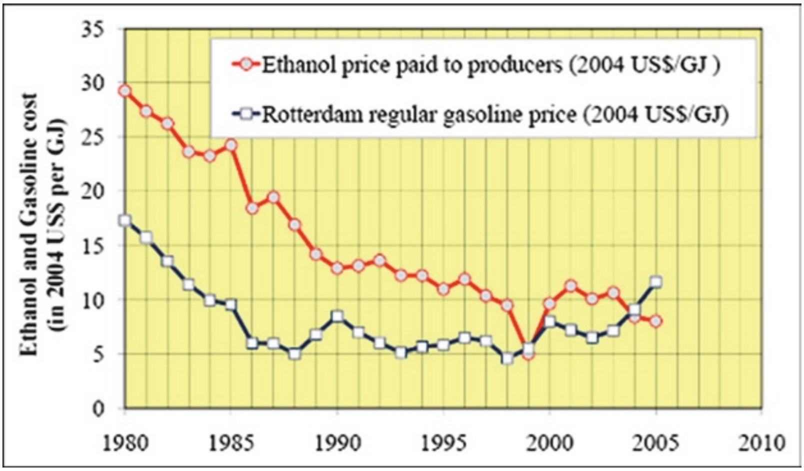

Fig. 5 The economic competitiveness of alcohol fuel compared to gasoline (Goldemberg et al. 2004)

large cities; examples are Beijing, Mexico city, São Paulo, and even Los Angeles. In the city of São Paulo, the quality of the air has improved remarkedly with the replacement of gasoline by ethanol, which today represents more than 50% of the fuel used by automobiles (CETESB 2008).

Emissions from land-use changes (including massive deforestation) could be a source of greenhouse gas emissions, as demonstrated by Fargione et al. (Fargione et al. 2008), but their study refers to a worst case scenario, which is not taking place presently, since expansion in the area used by biofuels is not taking place in virgin tropical forests. Such practice, of course, would release a large amount of CO_2, but extensive studies have been made on CO_2 releases, resulting from other agricultural practices that do not involve deforestation with results much less alarming.

There are almost 100 countries producing sugarcane in an area of 20 million hectares (approximately 0.5% of the world total area used for agriculture) (FAOSTAT 2007). The 15 most important producers representing 86% of total production of sugarcane. It is easy to convert plants producing sugar to ethanol distilleries, and most of the existing plants in Brazil have a dual purpose.

It is clear therefore that the production of ethanol from sugarcane could be expanded significantly if the example of Brazil is followed by several others using a fraction of the sugarcane for ethanol.

Ethanol can be produced from several feedstocks such as corn and other grains (mainly wheat), but the problem is the cost (Fig. 6).

Since the cost of production of ethanol from grains (in the US and Europe) is considerably higher than its cost of production from sugarcane (in Brazil); high import duties were imposed on ethanol imports in the US and Europe to protect local industries, which are therefore heavily subsidized. Table 2 gives estimates of

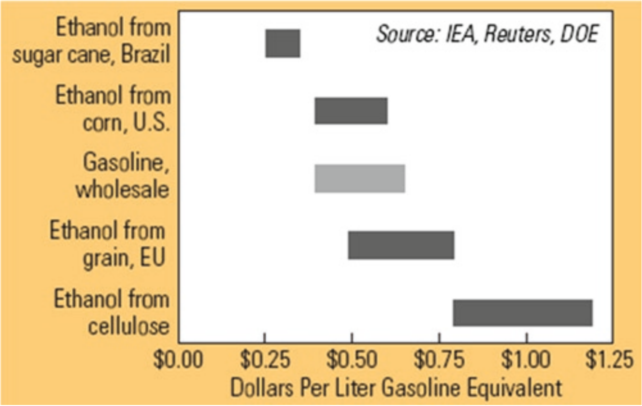

Fig. 6 Cost ranges for ethanol and gasoline production, 2006 (World Watch Institute 2006)

Table 2 Subsidies for biofuels in the US and EU 2006

	Ethanol			Biodiesel		
	Total billion	US$/liter	Billion liters	Total billion	US$/liter	Billion liters
Unites States	5.8	0.28	20.7	0.53	0.55	0.96
European Union	1.6	1.0	1.6	3.1	0.70	4.43
Total	7.4	–	22.3	3.63	–	5.39

the subsidies in the US and the European Union, which reached almost 12 billion dollars in 2006.

The removal of such subsidies is under discussion in the Doha round of negotiations, but prospects for progress in this area are not very good although countries such as France have decided to phase them out by 2012.

One of the reasons for the advantage of sugarcane is that all the energy needed for the processing comes from the bagasse which is not available using grains as the feedstocks. In this case, energy has to be "imported" by the distilleries most of which comes from fossil-derived fuels. This is the reason why the energy balance (i.e., the ratio of the energy contained in a litter of ethanol to the energy used in the process of preparation originating in fossil fuels) is 8:1 for sugarcane and 1.3:1 for corn. In a sense, ethanol from sugarcane is solar energy converted into a liquid while ethanol from corn is in reality fossil fuel (mainly coal in the US) converted into a liquid.

The consequence is that the greenhouse gas emissions resulting from the sugarcane route are much more favorable than from grains (Fig. 7).

6 Second Generation Technologies

However, progress in the use of cellulosic feedstocks of all kinds (including urban waste) using second generation technologies seems to be essential to broaden the feedstock used presently, which are in limited supply and could originate problems

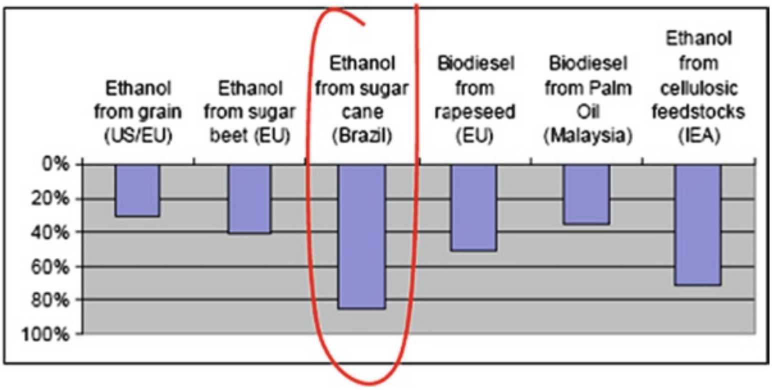

Fig. 7 Greenhouse gas reduction (Doornbosch and Steenblik 2007)

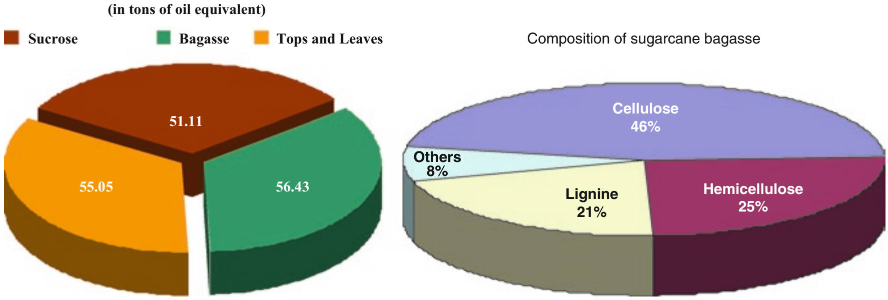

Fig. 8 Energy contained in 1,000 tons of sugarcane

such as a competition between fuels "versus" food. Excellent candidates for such feedstock are the bagasse of sugarcane and switchgrass.

In the case of sugarcane, bagasse contains a third of the energy contained in sugarcane, tags and leaves another third. With mechanized harvesting, which is progressing rapidly in Brazil, the available amount of such materials is increasing and is thus a prime candidate for second generation (Fig. 8).

Switch grass in the US seems to be an interesting option for the cellulosic route since it has a composition rather similar to bagasse.

Second-generation technologies that will allow the use of any cellulosic material for the production of biofuels are being actively pursued but have not yet reached commercial production.

This area is therefore open to new and creative approaches of great scientific technological and economic significance in the direction of replacing fossil fuels by renewable resources.

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Chapter 2
Bioenergy and the Sustainable Revolution

Wanderley D. dos Santos, Edgardo O. Gómez, and Marcos S. Buckeridge

1 Introduction

In this chapter, we will discuss some unexpected consequences that renewable energy policies might present for technological development and present an overview about the main current approaches to produce Biofuels. The technological barriers and alternatives investigated to overcome them are also discussed. In the first section, we argue that such radical changes in the way we think and sustain our development might imply that we are facing a new revolution in our energy production system. We proceed to elucidate some principles that are likely to determine the ideal and actual scenario of renewable fuels, including how ethanol can succeed and how biotechnological approaches chosen to produce second generation ethanol imply coping with the high complexity of lignocellulosic material. We also discuss the principles of biodiesel production, the importance of this incipient biofuel might offer to the setting of ethanol industry. Finally, we discuss the advantages and main perspectives in the short-term developments expected by the promising area of themochemistry to biofuel production.

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2 Energy Revolution

2.1 Mitochondrial Revolution

Evolution does not always occur as a soft continuum of myriads of little adaptations. It sometimes jumps. Since the origin of life around four billion years ago, green–blue bacteria increased the amount of molecular oxygen (O_2) in the atmosphere conspicuously. For most of the living organisms at that time (exclusively bacteria), oxygen was very dangerous. For some organisms it was (and still is) deadly. Thus, most bacteria lived only in oxygen-free environments. In the absence of oxygen, one of the main forms that heterotrophic organisms used to obtain energy was via the fermentation process. This process preserves part of the free energy content from a molecule of glucose in two adenosine-triphosphate (ATP) molecules, the standard energy fuel in catabolic processes. However, in this process, most of the chemical energy present in glucose is wasted as fermentation residues such as alcohol or lactic acid.

In the presence of oxygen, some organisms are able to accomplish cell respiration, a process in which glucose is completely oxidized to CO_2 and 36 ATP are produced from every single glucose molecule!. About two billion years ago, microorganisms undergoing selective pressure in an atmosphere that was becoming increasingly toxic with oxygen, developed the ability to tolerate and even obtain benefits from it. The last enzyme from the citric acid cycle to emerge and make possible respiration as we know it today was the α-cetoglutarate complex. This is thought to have occurred by mutations of genes of the pyruvate dehydrogenase complex, an enzyme complex with a similar structure and role in the citric acid cycle in aerobic as well as in anaerobic organisms (Fig. 1).

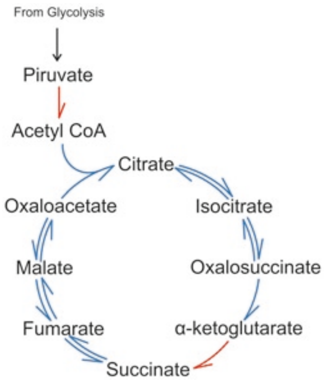

Fig. 1 Some anaerobic bacteria have the enzymes to produce several metabolic intermediates of the citric acid cycle. However, they cannot complete the cycle because they do not have the alpha-ketoglutarate dehydrogenase, which converts alpha-cetotarate into succinl-CoA (red arrow in the cycle). This enzyme probably evolved from pyruvate dehydrogenase. Such complex performs a similar reaction converting pyruvate into acetyl-CoA (red arrow above). Both complexes exhibit three analogous enzymes and use the same cofactors (TPP, lipoate, FAD, NAD, and coenzyme A).